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Research Article

Swertiamarin: An Active Lead from *Enicostemma littorale* Regulates Hepatic and Adipose Tissue Gene Expression by Targeting PPAR-γ and Improves Insulin Sensitivity in Experimental NIDDM Rat Model

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Enicostemma littorale (EL) Blume is one of the herbs widely used for treating and alleviating the effects of both type I and type II diabetes. However, lack of understanding of mechanism precludes the use of the herb and its molecules. In this study, we attempt to unravel the molecular mechanism of action of swertiamarin, a compound isolated form EL, by comparing its molecular effects with those of aqueous EL extract in alleviating the insulin resistance in type II diabetes. We further investigated hypolipidemic and insulin sensitizing effect of swertiamarin in experimentally induced noninsulin dependent diabetes mellitus (NIDDM) in rats. Swertiamarin (50 mg/kg) and aqueous extract (15 grams dried plant equivalent extract/kg) were administered to rats orally for 40 days and tight regulation of serum glucose, insulin, and lipid profile was found in both groups. Their mode of action was by restoring G6Pase and HMG-CoA reductase activities to normal levels and restoring normal transcriptional levels of PEPCK, GK, Glut 2, PPAR-γ, leptin, adiponectin, LPL, SREBP-1c, and Glut 4 genes. This suggests that both treatments increased insulin sensitivity and regulated carbohydrate and fat metabolism. This is the first report on the role of SM in regulating the PPARγ-mediated regulation of candidate genes involved in metabolism in peripheral tissues *in vivo*.

1. Introduction

Diabetes mellitus is the third most prevalent fatal disease in the world. Epidemiology shows that it is one of the major global health problems in the current scenario, targeting 6.4% of total world population. Around fifty million people are diabetic in Indian subcontinent; hence, India leads globally in this disease [1]. Indian population is more prone to diabetes than western population as their metabolism is quite different with lots of epigenetic modifications. Constant migration of people from rural to urban areas has contributed significantly in availability of food, calorie intake, and physical activities, which has major impact on the metabolic programming of an individual [2]. To understand the physiological and metabolic alterations of this disorder, many animal models are used. NA-STZ rat is a nonobese type 2 diabetes model that reflects the majority of diabetic patients among Asian races [3].

Type II diabetes mellitus is a heterogeneous metabolic disorder. Liver, skeletal muscles, and adipose tissues being insulin sensitive tissues show significant metabolic changes [4]. Insulin, apart from governing glucose uptake and metabolism, also influences the expression level of many genes related to energy metabolism [5].

Glut 2 plays a major role in glucose uptake and its metabolism in hepatic tissue, whose gene expression is influenced by glucose and insulin concentrations; hence, remarkable alterations in glucose would lead to significant change in the expression profile of this transporter. In diabetic condition, the expression of glucokinase (GK) enzyme is downregulated, which eventually leads to insulin resistance and hyperglycemia [6, 7]. PEPCK is a rate-limiting enzyme in gluconeogenesis; hence, its elevated expression leads to increased hepatic glucose production (HGP). The processes of glycolysis, gluconeogenesis, glycogenesis, and glycogenolysis are governed

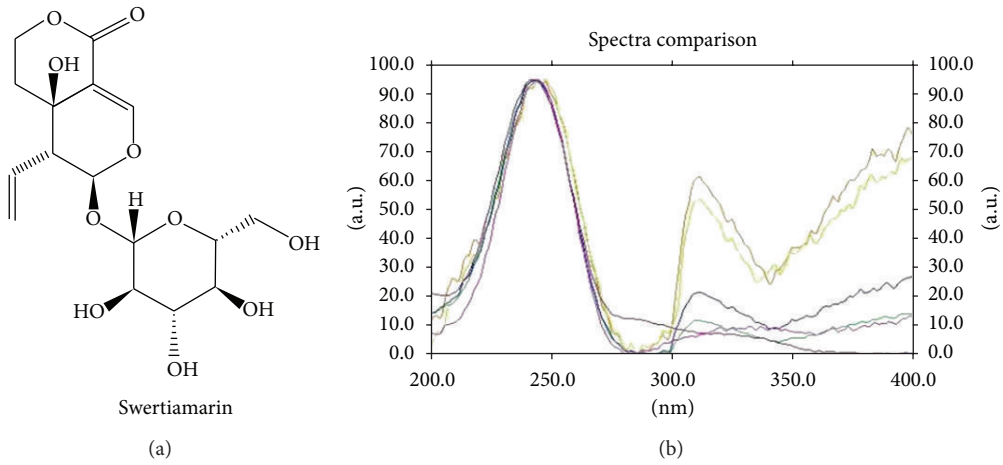

Figure 1: (a) Chemical structure of swertiamarin. (b) Overlay of ultraviolet absorption spectrum of swertiamarin isolated in lab and reference standard (λmax: 240–245 nm).

by insulin action, which are hampered due to altered action of insulin in TIIDM [8].

Obesity and dyslipidemia lead to the development of type II diabetes. Secretion of adipokines play a key role in controlling glucose and fat homeostasis of the entire body. PPAR-γ, playing a major role in regulation of transcription, is responsible for adipogenesis, mature adipocyte function, insulin signaling, carbohydrate metabolism, fat metabolism, and secretion of various adipokines like adiponectin, leptin, and so forth. Adiponectin positively and leptin negatively regulate insulin signaling in liver and muscle tissues [9]. Looking at the importance of this regulator, most studies have been directed toward developing synthetic PPARs ligands for insulin resistance and dyslipidemia in amelioration of TIIDM complications [10].

There are many treatments available ranging from synthetic drugs, like metformin, thiazolidinediones, GLP-1, DPP-4 inhibitor, and so forth, to herbal formulations like *Momordica charantia*, *Artemisia dracunculus*, *Gymnema sylvestre*, and so forth in amelioration of obesity and TIIDM [11]. In recent years, there has been renewed interest in the treatment of diabetes using herbal drugs, as World Health Organization (WHO) has recommended evaluation of the effectiveness of plants due to side effects of modern drugs [12]. Our lab has well documented *Enicostemma littorale* Blume owing antioxidant, hypolipidemic, and antidiabetic activities both in animals and human diabetic patients [13, 14]. Apart from antidiabetic activity, islet neogenic property of swertisin and normoglycemia in a diabetic rat are also reported [15]. Hence, it can be presumed that the EL extract potentially owes varied beneficiary activities due to the presence of many compounds within it. Swertiamarin (SM) is the major compound found in EL Blume and its pharmacokinetic study suggest that it is rapidly distributed in most of the tissues. Among all the tissues, the highest concentrations were found to be absorbed in the liver and its elimination was through kidney [16].

Many studies have been done to explore the mechanistic action of SM on TIIDM but are restricted to in vitro only. Also, other groups have focused on physiological and biochemical studies in neonatal-STZ models to understand role of SM for the treatment of obesity and dyslipidemia. However, the mechanism of action of SM has not been explored at a systemic level [17, 18].

Therefore, for the first time in this study we aim to assess the antidiabetic efficiency of SM in treating nicotinamide-streptozotocin (NA-STZ) diabetic rats and to elucidate its probable mechanisms of action. The current study was designed to answer the key question, "what is the mechanism of SM in regulating the expression levels of the candidate genes involved in carbohydrate, fat metabolism and insulin signaling in the liver and adipose tissue in TIIDM?"

2. Materials and Methods

2.1. Plant Material, Preparation of Aqueous EL Extract, and Isolation of Swertiamarin. The plant material of dry *E. littorale* was procured from Saurashtra region, Gujarat, India, during the month of August. Specimen was authenticated at Botany Department, M. S. University, Baroda with Voucher Specimen number [Oza 51,51(a)] deposited at the Herbarium of Botany Department, M. S. University, Baroda. Whole plant material was cleaned and dried. The fine powder of 40–60 mesh particle size was prepared in an electric grinder. The powder was soaked in thrice the amount of water for 2 hours and then boiled for 30 minutes. Three such extractions were done from each batch. Residue was removed by filtration, and water-soluble filtrate was pooled and evaporated to obtain extract concentration of 1 g dry plant weight equivalent per mL as per the method described above [19]. The yield of dry EL extract was found to be 28% (w/w). Isolation and characterization of swertiamarin from *E. littorale* were carried out by recording melting point and UV spectrometry with the standard sample of swertiamarin (Figure 1(a)). Purity of the sample was checked by HPTLC on ethyl acetate: methanol : water (0.7 : 0.2 : 0.1) as a solvent system [20].

2.2. Animals and Housing. Male *Charles Foster* rats housed at animal house facility of Department of Biochemistry were used for the study with *ad libitum* access to water

Table 1: List of primer sequences of RT-PCR with its amplicon size.

Gene	Accession number	Sequence forward primer $5'\rightarrow3'$	Sequence reverse primer $5'\rightarrow3'$	Product size
Glucokinase	NM_012565.1	AGTATGACCGGATGGTGGAT	CCGTGGAACAGAAGGTTCTC	139
Glut 2	NM_012879.1	CATTGCTGGAAGAAGCGTATCAG	GAGACCTTCTGCTCAGTCGACG	408
PEPCK	NM_198780	GTCACCATCACTTCCTGGAAGA	GGTGCAGAATCGCGAGTTG	84
Adiponectin	NM_144744.2	AATCCTGCCCAGTCATGAAG	CATCTCCTGGGTCACCCTTA	215
LPL	NM_012598.1	GAGATTTCTCTGTATGGCACA	CTGCAGATGAGAAACTTTCTC	276
Leptin	NM_013076.2	ACACCAAAACCCTCATCAAGA	GAAGGCAAGCTGGTGAGGA	184
SREBP-1c	XM_213329.4	GGCCTGCTTGGCTCTTCTC	GCCAGCCACAGCTGTTGAG	150
PPARγ	NM_013124	GGATTCATGACCAGGGAGTTCCTC	GCGGTCTCCACTGAGAATAATGAC	156
Glut 4	NM_012751.1	GCCTTCTTTGAGATTGGTCC	CTGCTGTTTCCTTCATCCTG	457
β-ACTIN	NM_031144	CCTGCTTGCTGATCCACA	CTGACCGAGCGTGGCTAG	505

and commercial chow (Pranav Agro Industries Ltd, Pune, India) in a well-ventilated animal unit (26–28°C, humidity 60%, 12 h light—12 h dark cycle). Care and procedures adopted for the present investigation were in accordance with the approval of the Institutional Animal Ethics Committee (938/a/06/CPCSEA, BC/14/2009-10). NIDDM rat model was developed by intraperitoneal injection of nicotinamide dissolved in normal saline at a concentration of 230 mg/kg body weight 15 minutes before giving an intraperitoneal injection of streptozotocin (Sigma, Aldrich) which was dissolved in 0.1 M citrate buffer (pH 4.5) at a concentration of 65 mg/kg body weight [21]. Hyperglycemia was confirmed by the elevated glucose level in fasting and postprandial blood sugar (PP_2BS) at 15–20 days of streptozotocin-nicotinamide injection.

2.3. Dosing of Swertiamarin and Aqueous Extract in NIDDM Rats. Swertiamarin and aqueous extract were orally administered for 40 days, and fasting serum glucose levels, OGTT profiles, and serum triglyceride levels were monitored. Rats were divided into five groups having six rats in each group; group I: normal control (NC), group II: DM, group III: DM + Aqueous extract (15 grams dried plant equivalent extract/kg b.w/day, p.o.), group IV: DM + swertiamarin (50 mg/kg/day, p.o.), and group V: DM + metformin (500 mg/kg b.w/day, p.o.).

2.4. Biochemical Parameters

2.4.1. Oral Glucose Tolerance Test (OGTT), Serum Insulin, and Lipid Profile. Rats were kept for overnight (10–12 hrs) fasting, and blood was collected from retro orbital sinus for estimation of fasting blood sugar. To measure OGTT of the rats, 2 gms/kg body weight of glucose was given orally and blood was collected at regular interval of every 30 min. till 2 hours. Serum was separated and glucose level was estimated using GOD-POD method by commercially available kit (Enzopak, India). Fasting serum insulin was estimated by rat insulin ELISA kit (Mercodia, Sweden). Total cholesterol, HDL-cholesterol, and TG was estimated using commercially available kits (Enzopak, India), and then the values of LDL-cholesterol and VLDL-cholesterol were derived from Friedewald's formula.

2.4.2. Determination of Liver Enzymes. Glucose-6-phosphatase was assayed according to the method of Koida and Oda, 1959, and the inorganic phosphorus (Pi) liberated was estimated by Fiske and Subbarow method, 1925. The ratio of absorbance of HMG CoA/absorbance of mevalonate was taken as an index of the activity of HMG CoA reductase activity required to convert HMG CoA to mevalonate, in the presence of NADPH [14].

2.4.3. RNA Isolation and Semiquantitative PCR. Animals from each group were sacrificed, and tissues (liver and adipocytes) were pooled. RNA was isolated from the homogenized liver and adipose tissue using the TRIzol reagent (Sigma Aldrich) as per manufacturer's instructions. A reverse-transcription reaction was performed using 2 μg RNA with MuLV reverse transcriptase in a 20 μL reaction volume containing DEPC treated water (Fermentas Kit). PCR product was amplified using gene-specific primers (Table 1). β-Actin was used as an internal control. The PCR products were analyzed by electrophoresis on 2.0% agarose gels or 15% DNA-PAGE, the gels were photographed after staining with ethidium bromide, and intensities of the band were calculated by densitometric analysis using the Image J software.

2.4.4. Immunoprecipitation and Immunoblotting for Insulin Signaling Proteins. Tissues were collected, suspended in lysis buffer containing 1X protease inhibitor cocktail, and homogenized. After centrifugation at 16000 g for 15 min. at 4°C, the supernatant was collected. Total protein content was quantified using Bradford assay (Biorad Bradford Solution, USA). Immunoprecipitation with insulin receptor (anti-IRβ 1 : 50) was performed using dynabeads G-protein IP kit (Invitrogen). Protein was loaded on a 10% SDS-polyacrylamide gel and then electrophoretically transferred onto a nitrocellulose membrane (GE Healthcare). The membrane was then incubated for 1 h at room temperature in blocking buffer (TBS-T containing 5% skimmed milk) and further incubated overnight with the primary antibodies for insulin receptor (1 : 1000), p-Tyr (1 : 1000), and PI(3)K (1 : 1000) at 4°C. Membrane was then washed four times with TBS-T and incubated with HRP-conjugated secondary antibodies (1 : 2500) for 1 h. Finally, membrane was developed and visualized with enhanced chemiluminescence western blotting detection system (Millipore Inc. USA).

2.5. Statistical Analysis. The results were analyzed using one-way analysis of variance (ANOVA) and student's t-test to determine the level of significance. $P < 0.05$ was considered to be significant. Results were expressed as mean ± SEM. The statistical analysis was carried out by using the Graph Pad Prism 3.0 software.

3. Results

3.1. Isolation and Confirmation of Swertiamarin from Enicostemma Littorale Blume. The n-butanol fraction yielded 7.31% w/w of swertiamarin. HPTLC densitogram confirmed identity of compound with standard swertiamarin as well as established purity of the same (Figure 1(b)). Ultraviolet absorption spectrum showed λmax in the range of 240–245 nm. Melting point of the compound was 190–192°C. The mass fragmentation pattern of compound represented base peak m/z of 374 representing molecular weight and m/z of 212 (M-162) a characteristic peak after removal of sugar moiety from the compound.

3.2. Swertiamarin Positively Regulates Various Physical and Biochemical Parameters in TIIDM. Decrease in body weight is a characteristic hallmark of TIIDM that happens due to the loss of the stored energy reserves. A classical way of determining the efficacy of drug treatment is the ability to restore body weight. As expected, we found a drastic decrease in the body weight in NA-STZ-induced diabetic rats as compared to the controls. The animals treated with standard drug metformin (MFO), aqueous extract, and SM showed significant increase in the body weight, which indicated reversal of DM condition (Figure 2).

Further, in an attempt to confirm the NIDDM condition in the animal model, oral glucose tolerance test was performed to ascertain severity of the diabetic condition. Our observation is in agreement with the known facts: the diabetic rats have a high PP_2BS and showed glucose intolerance as compared to the control rats (Figures 3(a) and 3(b)). MFO effect, as expected in Type II diabetes, reduced the PP_2BS in our rats (Figures 3(a) and 3(b)). The aqueous extract and SM-treated diabetic rats were observed to be normoglycemic.

Hyperinsulinemia is a characteristic feature of type II DM. However, in our DM group of rats, the serum insulin levels were lower than those in the control rats which matched with the reported model where insulin content was reduced up to 40% [21] (Figure 3(c)) and which mimic the later stage TIIDM. The EL extract and SM treatments were not capable of significantly ameliorating the hypoinsulinemic condition by increasing the serum insulin levels.

3.3. Swertiamarin Reduces Glucose 6 Phosphatase Activity. Changes due to diabetes are not only seen at the mRNA level but also at the protein levels. Many of the enzyme activities are altered in the peripheral tissues of diabetes. G-6-Pase is the key enzyme of gluconeogenesis in hepatic tissue. Its activity increases under diabetic condition due to deficiency of insulin or insulin action. The EL extract and SM restored elevated specific activity of G-6-Pase to normal levels (Figure 4).

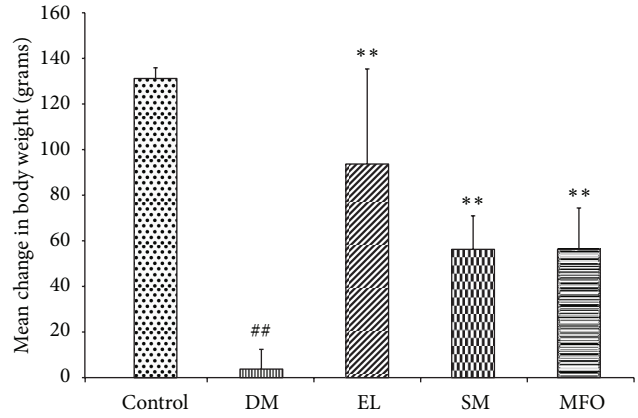

Figure 2: Change in body weight upon induction of diabetes and treatment of diabetic rats with EL, SM, and standard drug MFO. Data presented as mean ± SEM of 6 independent observations. $^{\#\#}P < 0.05$ versus control rats; $^{**}P < 0.05$ versus Diabetic rats.

3.4. Swertiamarin Regulates the Expression Levels of Candidate Genes of Carbohydrate Metabolism in TIIDM. Diabetes affects the expression of many candidate genes in the insulin dependent peripheral tissues like liver, adipose, and skeletal muscles. Glut transporters are the main glucose transporters in different peripheral tissues. Glut 2 is present in liver and is insulin independent. In diabetic rats, Glut 2 expression decreased significantly as compared to the control rats. We observed that the two treatments, EL extract and SM, rescue this decrease in expression, and the extract was more efficacious than the compound. PEPCK and glucokinase are the main enzymes of gluconeogenesis and glycolysis, respectively. They are regulated by insulin at the transcriptional level. In the diabetic condition, PEPCK has increased expression, while GK has decreased expression in liver. Treatments for diabetes should thus decrease the expression of PEPCK and increase the expression of GK. It was observed that EL extract and SM showed this (Figures 5(a) and 5(b)).

3.5. Swertiamarin Regulates the Altered Expression of Insulin Signaling Proteins in Liver. The liver homogenate was subjected to immunoprecipitation and immunoblotting with antiphosphotyrosine and anti-insulin receptor antibody. There was a decrease in the protein expression of insulin receptor in the diabetic group as compared to control. However, treatment with SM and EL extract restored not only the level of insulin receptor protein but also increased its phosphorylation. PI(3)K is a molecule downstream to insulin receptor, which gets recruited via IRS signaling pathway (Figures 6(a) and 6(b)).

3.6. Lipid Profile. We observed a significant increase in the serum triglyceride levels in the diabetic rats as compared to the control rats. This observation assertively showed that the serum triglyceride levels increase due to peripheral insulin resistance. Metformin (MFO) did not bring down the serum triglycerides levels significantly. But EL extract and its compound, SM, had a higher efficacy and reduced the serum triglyceride level near to control levels, thus making them out

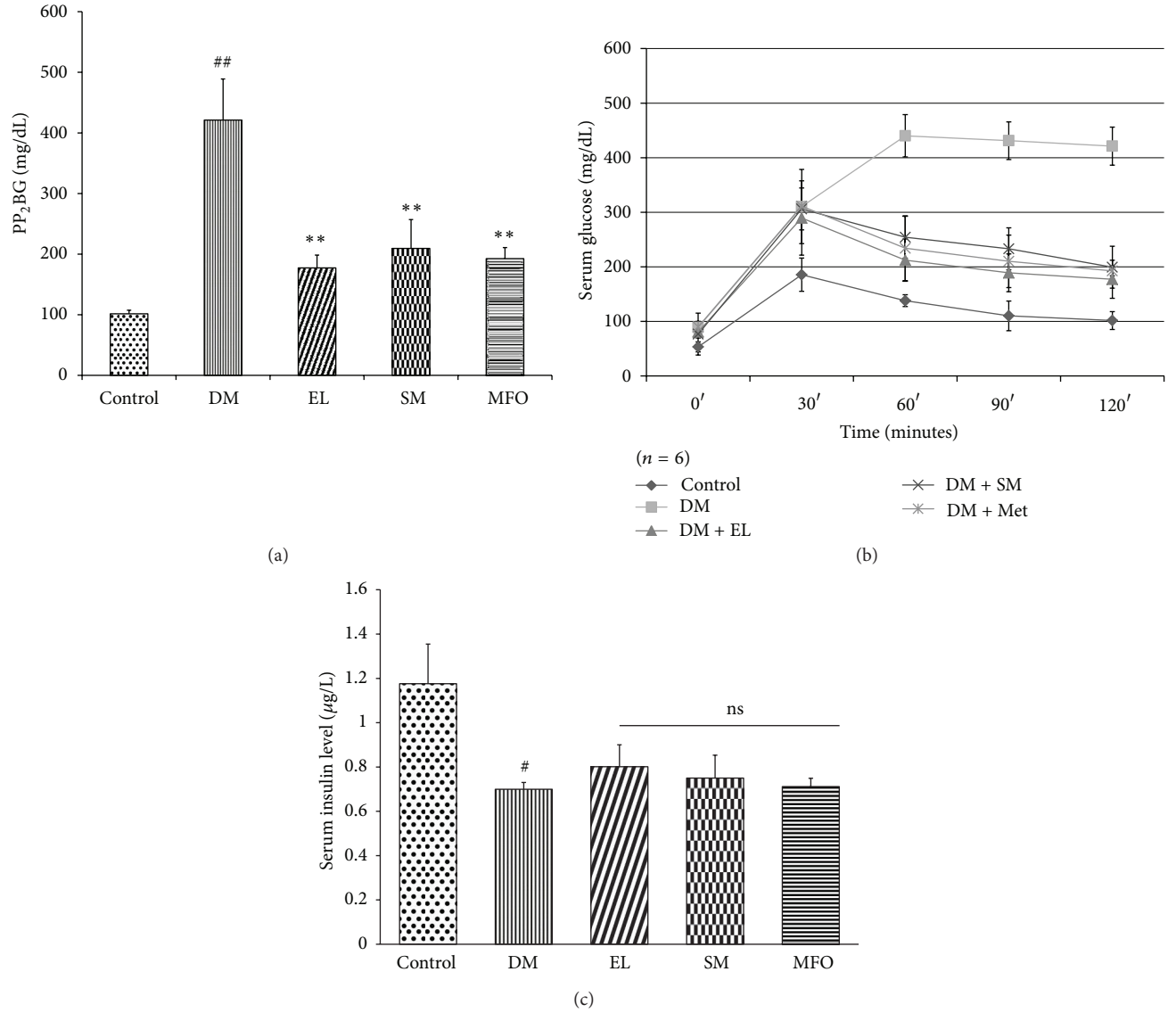

Figure 3: (a) Effect of EL extract, SM, and MFO treatments for 40 days on the postprandial serum glucose levels in diabetic conditions. Serum glucose levels were measured using GOD-POD. Data presented as mean ± SEM of $n = 6$ independent observations. $^{\#\#}P < 0.05$ versus control rats; $^{**}P < 0.05$ versus diabetic rats. (b) Effect of EL extract, SM, and MFO treatments for 40 days on the OGTT profile in diabetic conditions. Serum glucose levels were measured using GOD-POD. Data presented as mean ± SEM of $n = 6$ independent observations. (c) Effect of EL extract, SM, and MFO treatments for 40 days on the serum insulin levels in diabetic conditions. Serum insulin levels were measured using ELISA kit. Data presented as mean ± SEM of 4 independent observations. $^{\#}P < 0.05$ versus control rats. P value ns versus diabetic rats.

to be a safer alternative than the available anti-diabetic drugs. Aqueous extract and SM both were able to decrease serum cholesterol, serum LDL, and VLDL levels and increase HDL-cholesterol (Table 2).

3.7. Swertiamarin Regulates Activity of HMG-CoA Reductase Enzyme of Cholesterol Biosynthesis. HMG-CoA reductase is the major regulatory enzyme of cholesterol biosynthesis in the liver. An estimate of the enzyme activity can be used as a measure of the severity of the diabetic condition. The results of the present study showed inhibition of the HMG-CoA reductase activity in the diabetic rats treated with SM and EL extract as observed by higher HMG-Co/mevalonate (substrate/product) ratio compared to that of the diabetic control rats (Figure 7), thus supporting earlier reported hypolipidemic activity of SM.

3.8. Swertiamarin Regulates the Expression Levels of Various Key Enzymes of Lipid Metabolism and Glucose Transporter. Glut 4, an insulin dependent glucose transporter present in adipocytes and skeletal muscles, has decreased expression in diabetic rats due to increased insulin resistance. The key regulators of fat metabolism like adiponectin, SREBP-1c, PPAR-γ, and lipoprotein lipase 1 (LPL 1) are also found to be downregulated, while leptin is upregulated. The treatments with EL extract and SM helped in overcoming the insulin resistance by restoring the above gene expressions to normal levels (Figures 8(a), 8(b), and 8(c)).

Table 2: Lipid profile of control and treated diabetic rats.

Groups	Control	DM	DM + EL	DM + SM	DM + metformin
Triglyceride[#]	55.86 ± 4.69	110.94 ± 14.32[*]	49.63 ± 6.37[a]	63.34 ± 7.86[b]	94.54 ± 19.31
Total cholesterol[#]	88.55 ± 7.28	123.26 ± 15.80[*]	95.15 ± 10.88[a]	86.75 ± 13.64[b]	106.78 ± 12.54
HDL-C[#]	55.86 ± 4.66	27.44 ± 8.27[*]	44.63 ± 5.49[a]	40.94 ± 6.77[b]	41.89 ± 4.69
LDL-C[#]	21.52 ± 1.93	73.63 ± 3.2[*]	40.59 ± 4.37[a]	33.14 ± 5.86[b]	45.99 ± 5.64[c]
VLDL-C[#]	11.17 ± 0.69	22.19 ± 4.76[*]	9.93 ± 1.3[a]	12.67 ± 1.01[b]	18.90 ± 2.31

[#]Units: mg/dL. Values are given as mean ± SEM from 6 rats in each group, [*]$P < 0.005$ compared to control and a, b, c compared to diabetic group.

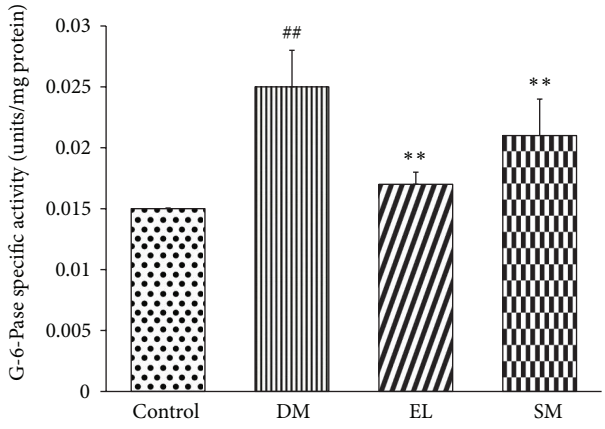

Figure 4: Effect of EL extract, SM, and MFO treatments for 40 days on the specific activity of G-6-Pase enzyme from hepatic tissue in diabetic conditions. It was assayed according to Koida and Oda method, and released Pi was estimated using Fiske-Subbarao method. Data presented as mean ± SEM of 5 independent observations. [##]$P < 0.05$ versus control rats; [**]$P < 0.05$ versus diabetic rats.

3.9. Expression of Insulin Signaling Proteins in Adipose Tissue. The adipose tissue homogenate was subjected to immunoprecipitation and immunoblotting with antiphosphotyrosine and anti-insulin receptor antibodies. There was a decrease in the protein expression of insulin receptor and PI(3)K in the diabetic group as compared to control (Figure 9(a)). Treatment with SM and aqueous extract restored the level of insulin receptor, IR phosphorylation, and PI(3)K protein level (Figure 9(b)).

4. Discussion

Obesity and insulin resistance are major causes of TIIDM. Multiple problems in diabetes lead to a cascade of complications in peripheral tissues. For controlling hyperglycemia, dyslipidemia, and insulin resistance, many synthetic drugs have been used. Also, the beneficiary effects of herbal extracts and compounds have been exploited [12].

Enicostemma litorrale Blume belonging to Gentianaceae family has been evaluated for its hypoglycemic, antioxidant, and hypolipidemic activities [13, 14]. Further, the author's research group is continuously involved in exploring the wide spectrum hidden potentials of this plant for islet neogenesis and various diabetic complications [15, 22, 23]. Qualitative analyses of EL have demonstrated the presence of flavonoids and secoiridoid glycosides. Swertiamarin, a secoiridoid glycoside, is one of the most valuable compounds that is present in abundance and possesses various therapeutic activities: antidiabetic, antinociceptive, antilipidemic, and anti-inflammatory [17, 24]. Hence, it is interesting to unravel the mechanism of this compound's action against the development and progression of TIIDM.

Various animal models are available for studying TIIDM. NA-STZ nonobese NIDDM rat model was selected for this study that best mimics the non-insulin dependent diabetes condition prevalent in humans [21]. Glucose intolerance, altered insulin content and skewed lipid profile of the experimental animals, resemble the hallmarks of this model that actually persist in a later stage of human TIIDM patients. Effect of SM in restoration of body weight, OGTT profile, and hypolipidemic activity on the experimental animals potentially proves the reported characteristics of this compound in the present study. Many herbal compounds are reported in regulating the expressions of the candidate genes involved in metabolic pathways and thus ameliorating TIIDM. This led to our interest in unraveling the molecular mechanism of aqueous extract and SM in restoring the altered expressions of the candidate genes involved in TIIDM.

Carbohydrate and fat metabolism regulation is governed mainly in insulin sensitive peripheral tissues like liver, muscle, and adipose tissue. Liver is the master organ in metabolism where glucokinase, PEPCK, and glycogen phosphorylase are rate limiting enzymes in glucose flux, gluconeogenesis, and glycogenolysis, respectively [8].

Decreased activity of G-6-Pase was observed in SM-treated diabetic rats, which correlate to the results reported by us previously in aqueous extract-treated diabetic rats. Reduction in the PEPCK gene expression was observed when diabetic rats were treated with SM in the current study. PEPCK expression restoration reflects increased insulin sensitivity [25–27]. Increased activity of this limiting enzyme leads to more hepatic glucose production (HGP), which worsens the diabetic condition. Glucose concentration increases the binding of SREBP-1c on promoter of Glut 2, increasing its transcription which is regulated by glucose and insulin. Expression levels of glucokinase and Glut 2 have been shown to be decreased in the hepatocytes of the diabetic rats [28]. In agreement with earlier reports, our results show that diabetic rats have decreased glucokinase and Glut 2 expressions which are reversed upon treatment with EL aqueous extract and SM.

It is well documented that diabetic patients exhibit dyslipidemia. Our lab previously reported a decrease in serum triglycerides, cholesterol, LDL, and VLDL with increased

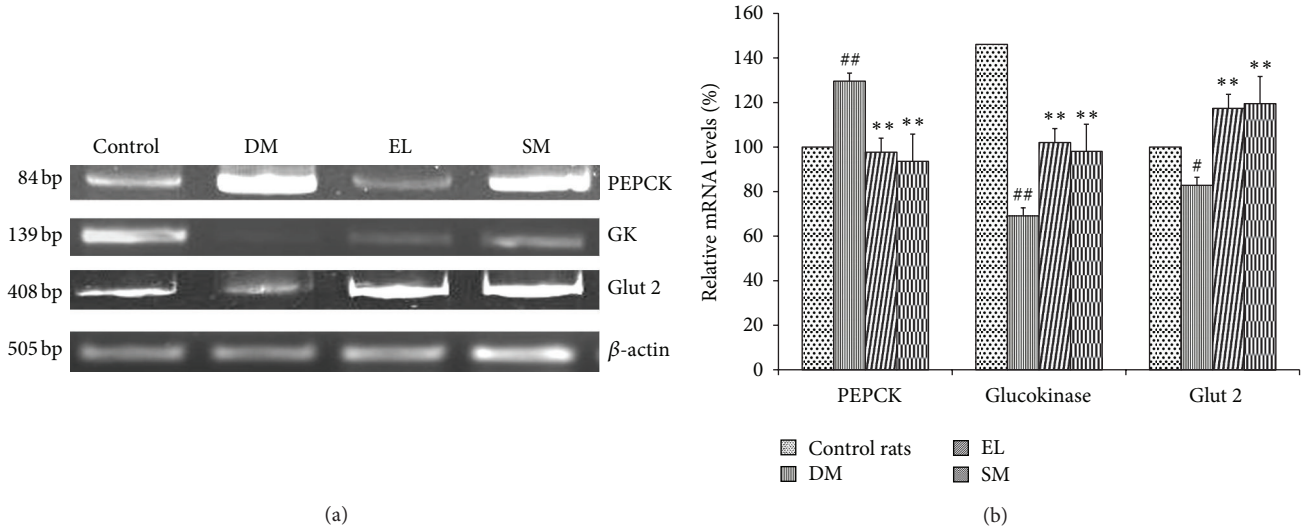

Figure 5: (a) Effect of EL extract and SM treatments on the mRNA expression of PEPCK, GK, Glut 2, and β-actin in the hepatic tissue as compared to diabetic rats (Gel image). (b) Effect of EL extract and SM treatments on the expression of PEPCK, GK, and Glut 2 in the hepatic tissue as compared to diabetic rats. The expression levels were checked using semi-quantitative RT-PCR and densitometric analysis. Data presented as mean ± SEM of 4 independent observations. $^{\#\#}P < 0.05$ versus control rats; $^{**}P < 0.05$ versus diabetic rats.

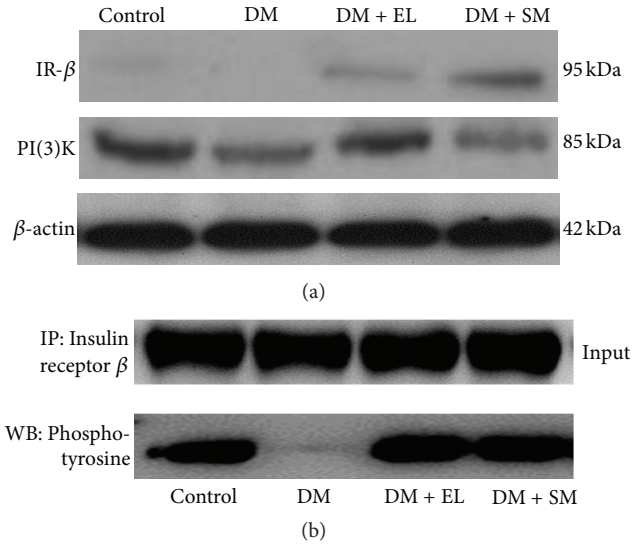

Figure 6: (a) Western blot study showing the effect of EL extract and SM treatments on the expression of insulin signaling proteins: IR and PI(3)K in the hepatic tissue as compared to diabetic rats. β-actin was taken as an internal control. (b) Immunoprecipitation study showing the effect of EL extract and SM treatments on the tyrosine phosphorylation of insulin signaling proteins: IR in the hepatic tissue as compared to diabetic rats (200 ug protein).

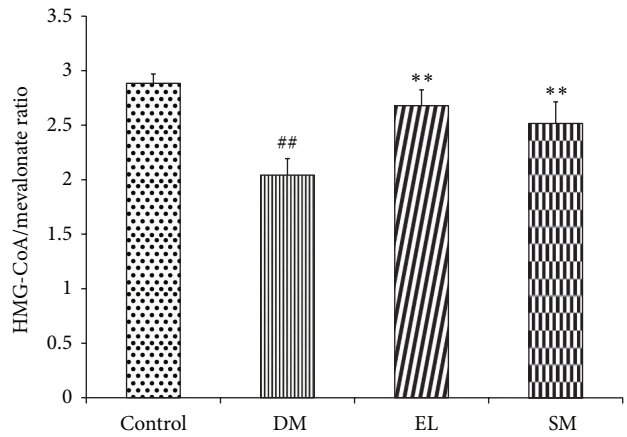

Figure 7: Effect of EL extract and SM treatments for 40 days on the ratio of absorbance of HMG CoA/absorbance of mevalonate was taken as an index of the of HMG CoA reductase activity from hepatic tissue in diabetic conditions. Data presented as mean ± SEM of 5 independent observations. $^{\#\#}P < 0.05$ versus control rats; $^{**}P < 0.05$ versus diabetic rats.

HDL level in aqueous extract-treated cholesterol fed rats [14]. SM is beneficial in bringing the lipid profile in neonatal-STZ rats to normal. [18]. HMG-CoA reductase is a key enzyme involved in the cholesterol biosynthesis in the hepatic tissue, which increases the free fatty acid level that leads to insulin resistance. SM and aqueous extract correct lipid profile and HMG-CoA reductase activity [29].

Adipose tissue plays an important role in fat metabolism. In TIIDM, increased lipolysis and decreased lipogenesis occur in liver and adipose tissues. Obesity decreases expression of lipogenic genes like SREBP-1c, PPAR-γ, and aP2, which causes increase in hepatic lipogenesis hence leading to a fatty liver [30]. PPAR-γ is a key transcriptional factor regulating the expression of SREBP-1c, leptin, adiponectin, and LPL. Low adiponectin and high leptin levels can cause insulin resistance in adipocytes thus leading to diabetes [31]. In the present study, aqueous extract and SM both regulate PPAR-γ mRNA levels in NA-STZ-induced diabetic rat model along with induced expression of adiponectin, LPL, and SREBP-1c suggesting it as a potent modulator of diabetes-related modification in adipocytes and thus corrects overall lipid

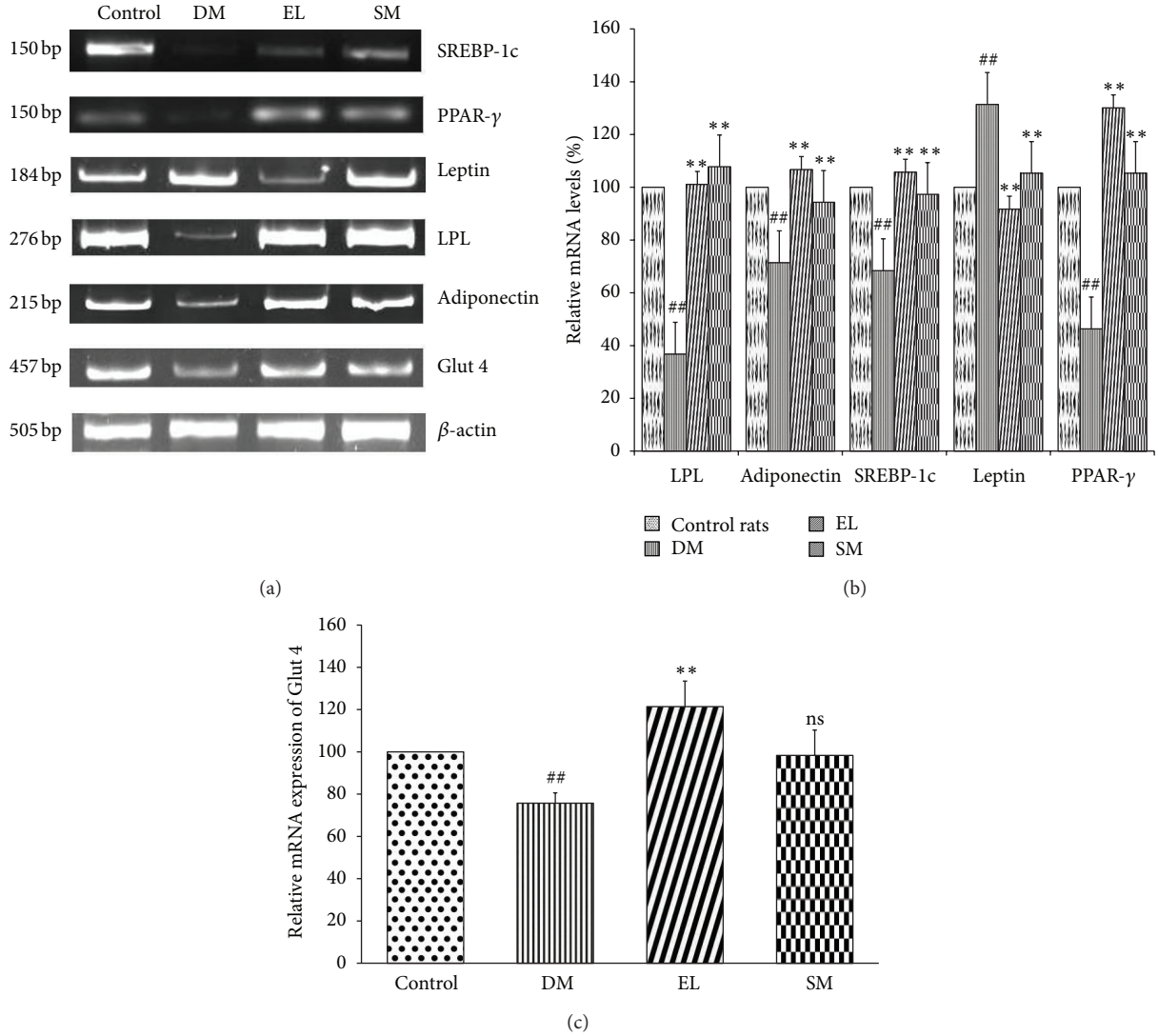

FIGURE 8: (a) Effect of EL extract and SM treatments on the mRNA expression of SREBP-1c, PPAR-γ, leptin, LPL, adiponectin, Glut 4, and β-actin in the adipose tissue as compared to diabetic rats. (Gel image). (b) Effect of EL extract and SM treatments on the expression of the major genes regulating the fat metabolism in the adipocytes as compared to diabetic rats. The expression levels were checked using semi-quantitative RT-PCR and densitometric analysis. Data presented as Mean ± SEM of 4 independent observations. $^{\#\#}P < 0.05$ versus control rats; $^{**}P < 0.05$ versus diabetic rates. (c) Effect of EL extract and SM treatments on the expression of Glut 4 in the adipocytes as compared to diabetic rats. The expression levels were checked using semi-quantitative RT-PCR and densitometric analysis. Data presented as mean ± SEM of 4 independent observations. $^{\#\#}P < 0.05$ versus control rats; $^{**}P < 0.05$ versus diabetic rates; P value ns versus diabetic rats.

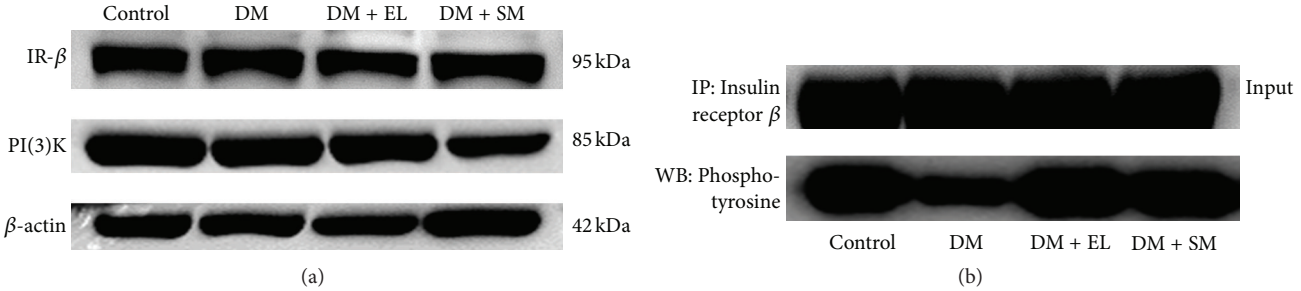

FIGURE 9: (a) Western blot study showing the effect of EL extract and SM treatments on the expression of insulin signaling proteins: IR and PI(3)K in the adipose tissue as compared to diabetic rats. β-actin was taken as an internal control. (b) Immunoprecipitation study showing the effect of EL extract and SM treatments on the phosphorylation of insulin signaling proteins: IR in the adipose tissue as compared to diabetic rats (100 ug protein).

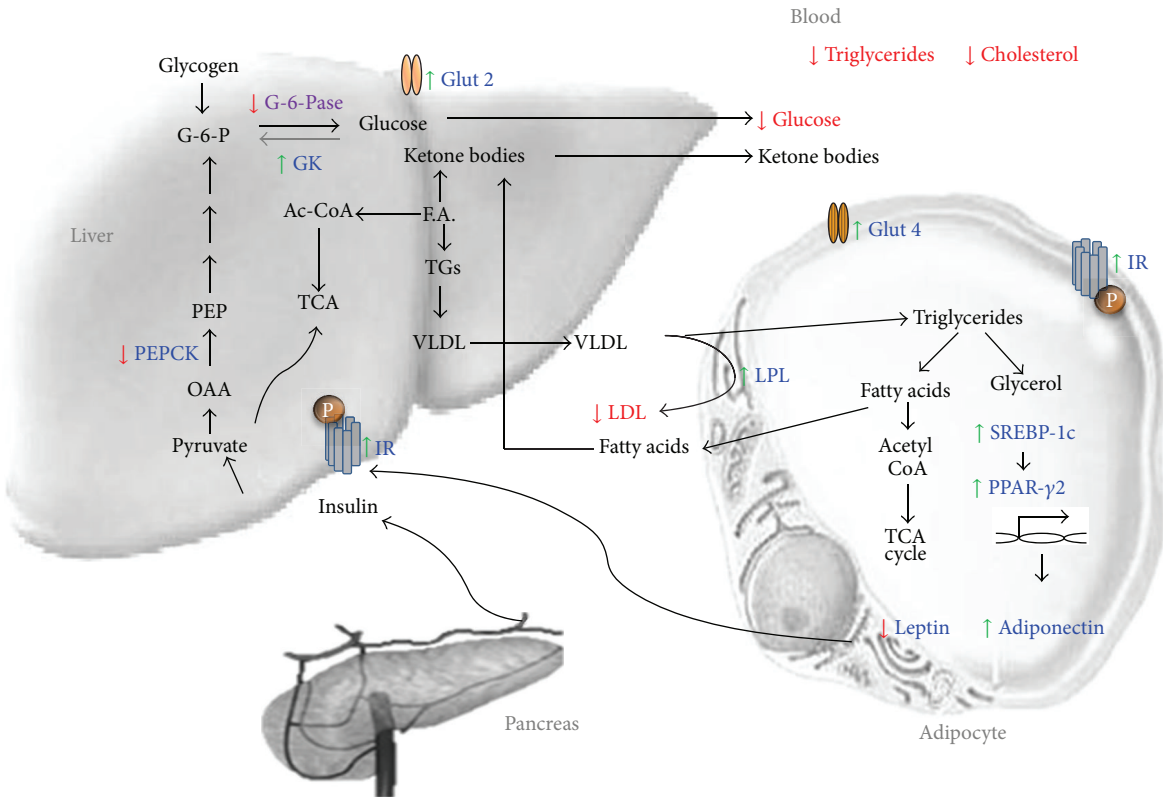

Figure 10: Schematic representation of swertiamarin in amelioration of insulin resistance and TIIDM. Figure shows carbohydrate and fat metabolic pathways and candidate genes which are altered during diabetes. Swertiamarin treatment modulates not only the expression of these target genes which is marked in blue but also the metabolite levels in blood marked in red.

metabolism, which can correct dyslipidemia by increasing insulin sensitivity [32].

Insulin sensitivity depends on the binding of insulin to its receptor, which autophosphorylates and further leads to downstream signaling cascade. Treatment of diabetic animals with aqueous extract and SM showed increased insulin receptor protein synthesis and its autophosphorylation in liver and adipose tissues, which improves insulin sensitivity in TIIDM. Phosphorylation of PI(3)K is mainly responsible for insulin stimulated glucose uptake by Glut 4, which is responsible for peripheral glucose disposition in muscle and adipose tissue. It has been reported that cinnamon extract improves insulin action and glucose uptake by enhancing the insulin signaling pathway in skeletal muscle [33].

The results of the current study proves that SM activates PPAR-γ and its regulatory genes, which improves fat metabolism in adipose tissue. By controlling PPAR-γ, SM can maintain the status of small adipocytes that reduces expression of leptin and TNF-α and increases expression of adiponectin. Increased Adiponectin secretion acts in an autocrine and paracrine manner, which improves expression of insulin receptor, its autophosphorylation, and downstream insulin signaling in liver as well as in adipose tissue [34]. This is the need of the hour, a drug which is able to maintain a balance between all the players involved in the carbohydrate and fat metabolism in the peripheral tissues (Figure 10).

5. Conclusion

The NA + STZ-treated rats show glucose intolerance, increased serum TG, and decreased serum insulin levels, indicating NIDDM-like condition. Treatment with aqueous EL extract and swertiamarin has been found to reduce the glycemic burden as monitored by OGTT profile. In diabetic rats, swertiamarin enhances insulin sensitivity resulting in restoration of altered gene expression of glucose metabolism in liver. In dyslipidemic condition, swertiamarin plays a crucial role in lowering surplus cholesterol by inhibiting HMG-CoA reductase activity. This is the first report *in vivo* that highlights a significant role of SM as a regulator of gene expression under the control of transcriptional factors like PPAR-γ, hence suggesting that SM improves insulin sensitivity and modulates carbohydrate and fat metabolism by regulating PPAR-γ. Present results thus strongly suggest that SM can be a potent therapeutic agent against TIIDM.

Abbreviations

DPP-4:	Dipeptidyl peptidase-IV
EL:	*Enicostemma littorale*
G-6-Pase:	Glucose 6 phosphatase
GK:	Glucokinase
GLP-1:	Glucagon-like peptide-1

Glut 2:	Glucose transporter 2
Glut 4:	Glucose transporter 4
HGP:	Hepatic glucose production
HMG CoA Reductase:	3-hydroxy-3-methyl-glutaryl-CoA reductase
HPTLC:	High performance thin layer chromatography
IR:	Insulin receptor
LPL:	Lipoprotein lipase 1
MFO:	Metformin
NA:	Nicotinamide
NIDDM:	Noninsulin dependent diabetes mellitus
OGTT:	Oral glucose tolerance test
PEPCK:	Phosphoenolpyruvate carboxykinase
PI(3)K:	Phosphatidylinositide 3-kinases
PP_2BS:	Postprandial (2 hours) blood sugar
PPAR-γ:	Peroxisome proliferator-activated receptor gamma
SM:	Swertiamarin
SREBP-1c:	Sterol regulatory element-binding protein-1c
STZ:	Streptozotocin
TG:	Triglycerides
TIIDM:	Type II diabetes mellitus
WHO:	World Health Organization.

Conflict of Interests

The authors declare that there is no conflict of interest associated with this paper.

Authors' Contribution

Sarita Gupta and Tushar P. Patel conceived and designed the experiments; Sanket Soni and Sarita Gupta were responsible for isolation and characterization of compound; Tushar P. Patel, Pankti Parikh, Jeetendra Gosai, and Ragitha Chruvattil performed other experiments; Tushar P. Patel and Sarita Gupta analyzed the data; Tushar P. Patel and Sarita Gupta wrote the paper.

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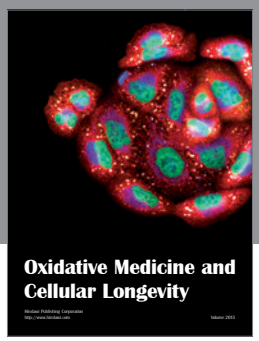

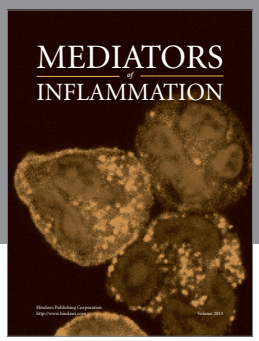

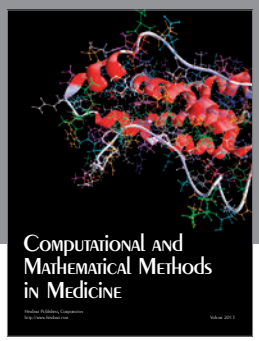

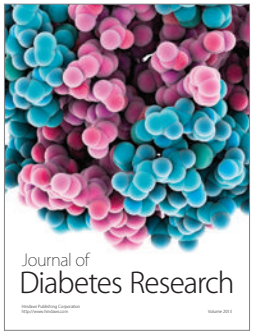

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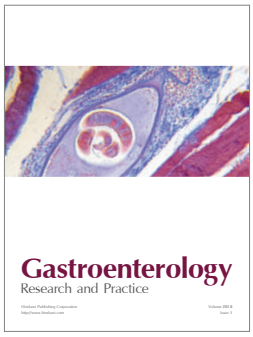

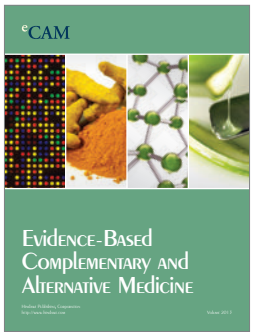

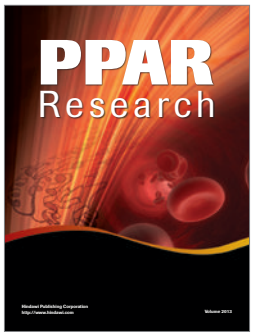

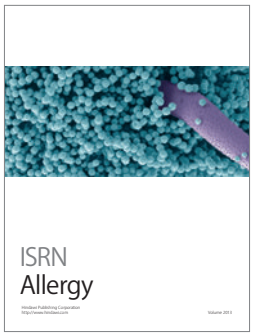

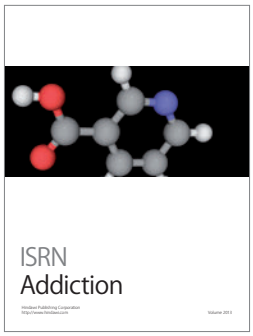

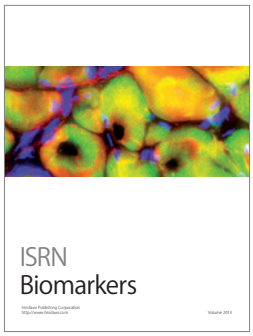

POSTERS

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CONFERENCES

Effect of the *Enicostemma littorale* Blume on gene expression of candidate metabolic regulators in NIDDM Rat hepatic tissue.

Patel Tushar,* *Parikh Pankti, Thakkar Nidhi, Soni Sanket and Gupta Sarita*

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ABSTRACT

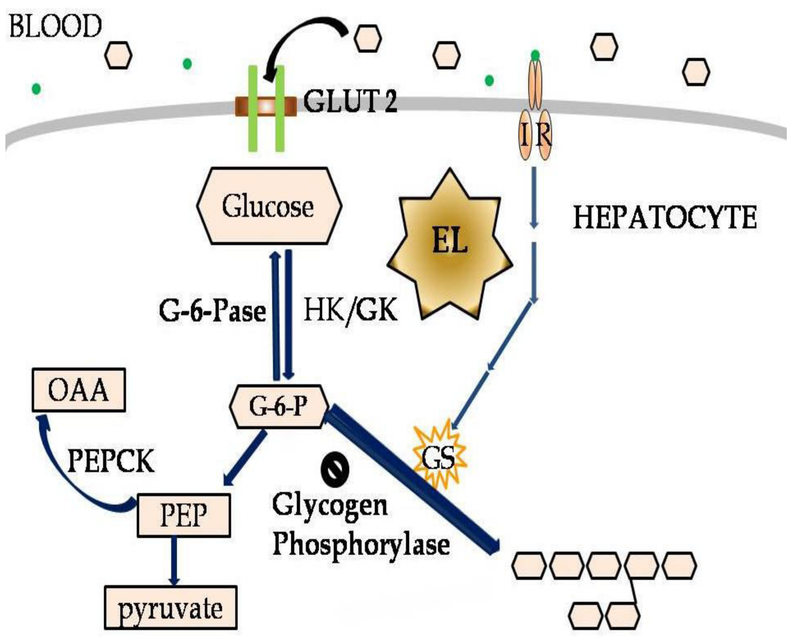
Diabetes mellitus is a multifactorial and complex metabolic disease. The pathogenesis of type 2 diabetes mellitus, resulting from defects in insulin receptor function, IR-signal transduction, carbohydrate or lipid metabolism contributes to insulin resistance in target peripheral tissues. These defects are targets of current pharmacological treatments as well as potential sites for new therapies. Large number of herbal extracts & active principal compounds demonstrated to have hypoglycaemic activity, offer valuable alternatives for control of the disease. *Enicostemma littorale* (EL) Blume belonging to family of Gentianaceae, being used by rural folk for the treatment of diabetes. The overall aim of this work is to elucidate the molecular mechanism of EL extract in ameliorating type 2 diabetes. For this, a NIDDM rat model was developed using niacinamide and Streptozotocin. The model showed hyperglycemia, glucose intolerance and signs of later stage of type 2 diabetes. Alteration of expression of various metabolic enzymes and glucose transporter underlies these changes. The effects of EL extract on this altered expression pattern in diabetic rats were checked. Aqueous EL extract efficiently restored expression levels of metabolic enzymes like PEPCK and Glucokinase to near normal levels. The restoration of GLUT-2 expression in EL treated diabetic rats was striking. These results suggest that EL increases insulin sensitivity either by potentiating insulin signaling or regulating some key transcription factors like SREBPs, PGC, CREBP and FoxO protein.

INTRODUCTION

Enicostemma littorale Blume is used as a herb for eons by the tribal people of Gujarat.

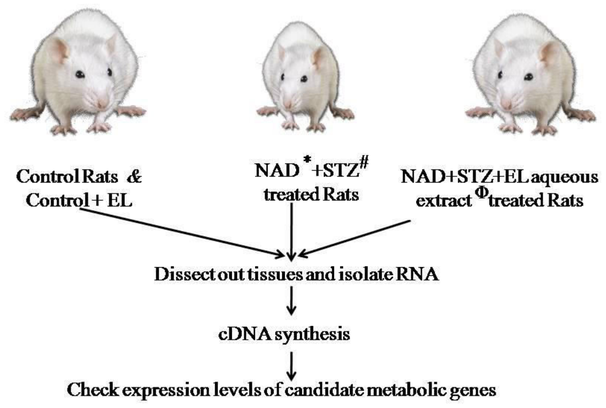
It has been shown that aqueous extract of *Enicostemma littorale* Blume has anti-diabetic activity in the alloxan induced diabetic rats and even in the NIDDM human patients. ***(Maroo J et al., 2002; Vasu V T et al., 2003; Gupta S. et al., 2005., Vasu V T et al., 2005)*** Similar effect has been shown in the Streptozotocin induced NIDDM rats.***(Goyal R.,et al., 2003)*** Aqueous EL extract has been shown to have many phenolics, alkaloids, flavonoids, xanthones and glycosides, which in other herbs have been shown to alleviate diabetic conditions and complications. ***(Gupta S et al., 2005)*** In NIDDM condition, where insulin action is impaired, EL extract administration will alter the expression of the genes **(PEPCK, Glycogen Phosphorylase, GLUT-2 and Glucokinase)** involved in carbohydrate metabolism in the Niacinamide + Streptozotocin induced NIDDM rats.. The overall aim of this project is to elucidate the molecular mechanism of EL extract in ameliorating the NIDDM condition and its role in improving the condition of Type 2 Diabetes.

HYPOTHESIS



➢**Aqueous EL extract** was prepared according to ***Maroo J et al.,*** 2005.
➢ Average yield : 28-30 %

PLAN OF WORK

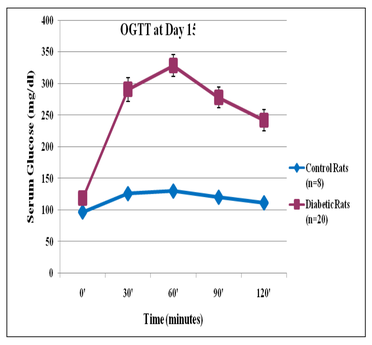


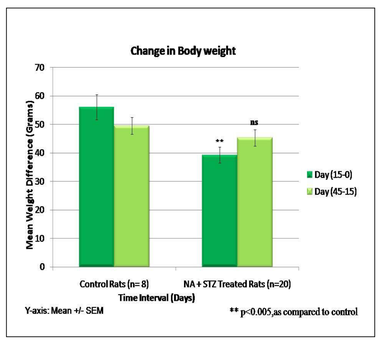
Genes : PEPCK,Glycogen Phosphorylase, Glucokinase, GLUT-2
β-actin as the internal control.
* 230 mg/kg b.w ; # 65 mg/kg b.w ; Φ 15 g Equivalent extract/kg b.w

Adult males 150-225 g (n=6 each group)

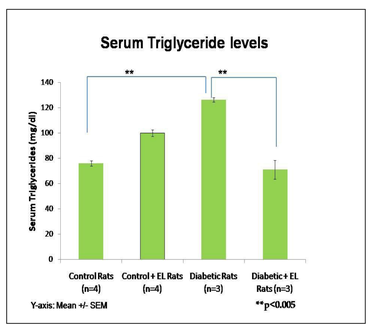
RESULTS

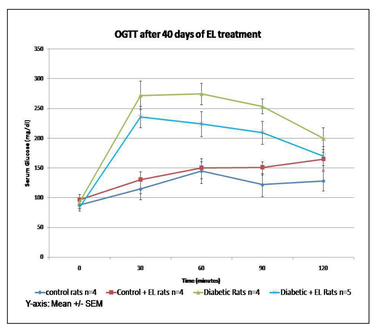
Confirmation of Diabetes





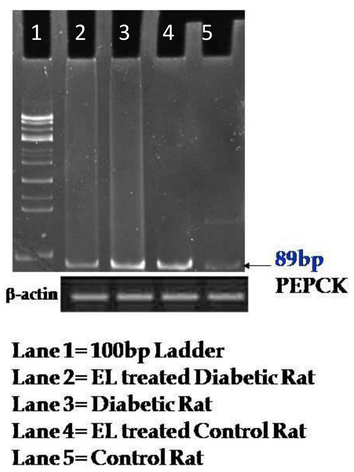
Effect of EL Aqueous extract

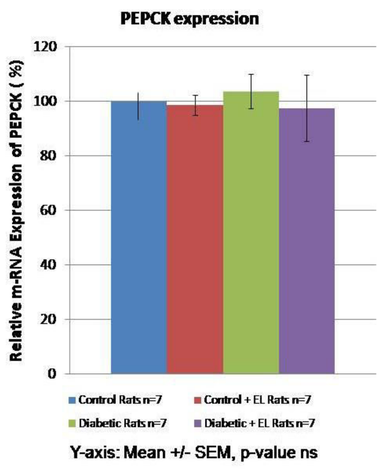




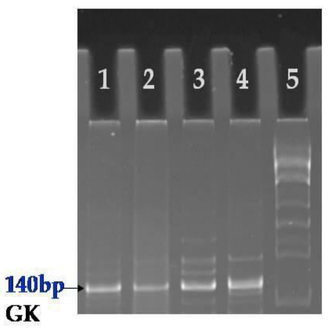
Gene Expression levels after EL extract administration

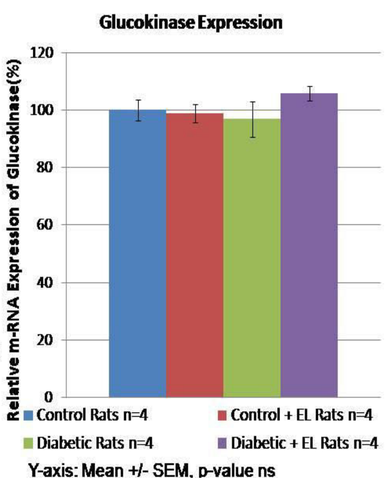
m-RNA expression of PEPCK



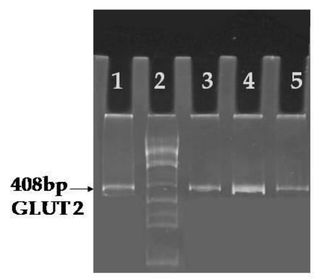


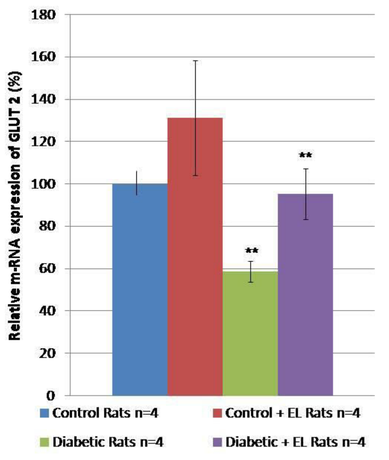
m-RNA expression of Glucokinase





m-RNA expression of GLUT-2





DISCUSSION

- The NA + STZ treated rats show mild to moderate increase in glucose intolerance as observed with the OGTT profiles indicating NIDDM like condition.(5)
- Serum Triglyceride profile at 6 weeks confirms that the model is an NIDDM model. Though it should be further confirmed by estimating Serum Insulin levels.(10)
- The decrease in Serum Triglyceride levels after EL extract treatment also support the hypolipideamic activity of EL extract.(2,4,13)
- PEPCK expression increases in diabetic rats while Glucokinase expression decreases.(7,8,14)
- RT-PCR analyze showed marginal change in the gene expression of both PEPCK & GK which can be further quantified by Real Time PCR (3,11)
- GLUT 2 expression decreases quite significantly in the diabetic rats showing that the insulin action is impaired in these animals.
- GLUT 2 expression is increased by EL extract in the diabetic rats. The results show a trend in the expression levels.

CONCLUSION

NIDDM rat model show impaired glucose tolerance even after ~6 weeks of NA + STZ treatment shows that the model is stable for a long time period. The biochemical parameters confirm the NIDDM like condition simulated in the model. The changes in gene expression also conform to the reported and expected results. EL extract is able to bring the expression back to the normal levels in the diabetic rats. Thus, it can be said that EL extract in its crude aqueous form shows its effect at a particular gene expression level via mimicking insulin or at the level of insulin signaling or by activating certain nuclear transcription factors

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Swertiamarin, a bitter *secoiridoid glucoside* improves fat metabolic genes, cellular antioxidants and ameliorate insulin resistance in oleic acid induced hepatic steatosis in HepG2 cells

DBT-MSUB-ILSPARE PROJECT

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ABSTRACT

Hepatic lipid accumulation, inflammation and insulin resistance contribute to non-alcoholic fatty liver disease (NAFLD). We hypothesized that hypolipidemic and antioxidant activity of swertiamarin would attenuate events leading to hepatic steatosis and insulin resistance. Steatosis was induced in HepG2 cells by supplementing 1 mM oleic acid (OA) in the culture media for 24 h. OA induced hepatic steatosis in HepG2 cells was marked by significant accumulation of lipid droplets as determined by Oil-Red-O (ORO) based colorimetric assay, increased triacylglycerol (TAG) and decreased % LDH release activity. Swertiamarin (25 µg/ml) decreased TAG content by 2 folds and was effective in reducing LDH release(50%). OA induced insulin resistance was evident by inhibition of insulin signalling and glucose uptake. Swertiamarin reduced insulin resistance and improved sensitivity by restoring the level of insulin receptor, Akt phosphorylation, PPAR-γ and PI(3)K proteins. In addition, Real Time-PCR results confirmed OA up-regulated sterol regulatory element binding protein-1(SREBP-1) and fatty acid synthase (FAS), resulting in increased fatty acid synthesis. Whereas PPARγ which is important for triglyceride synthesis and carnitine palmitoyltransferase 1(CPT-1) which governs fatty acid oxidation system in the mitochondria were up-regulated in OA model and was restored by treatment with Swertiamarin. Hence, swertiamarin effectively reversed NAFLD symptoms by decreasing triglycerides accumulation, fatty acid synthesis, insulin resistance and increasing cellular antioxidants in OA induced hepatic steatosis in HepG2 cells.

INTRODUCTION

➢Defining Nonalcoholic Fatty Liver Disease: A liver biopsy showing moderate to gross macrovesicular fatty change with or without inflammation (lobular or portal), Mallory bodies, fibrosis or cirrhosis.

➢NAFLD Spectrum of Disease : Steatosis > Steatohepatitis (NASH) > NASH with Fibrosis > Cirrhosis

➢Epidemiology : Prevalence of NAFLD 13-18% and that of NASH specifically 2-3% (1.2-9%). Prevalence of NAFLD in Indian population is 5 – 28% among Asian population.

➢Risk Factors : Classic TRIAD → Obesity, Diabetes and Dyslipidemia

➢Pathogenesis : "2 Hit" Paradigm

➢"First hit" – Excess fat accumulation and insulin resistance

➢"Second hit" – Intrahepatic oxidative stress, Lipid peroxidation, TNF-alpha and cytokine cascade

➢Oleic acid -induced steatosis in HepG2 cells is vitro model of steatosis is critical in understanding the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and searching for effective therapies.

➢*Enicostemma littorale* Blume is used as a herb for eons by the tribal people of Gujarat.

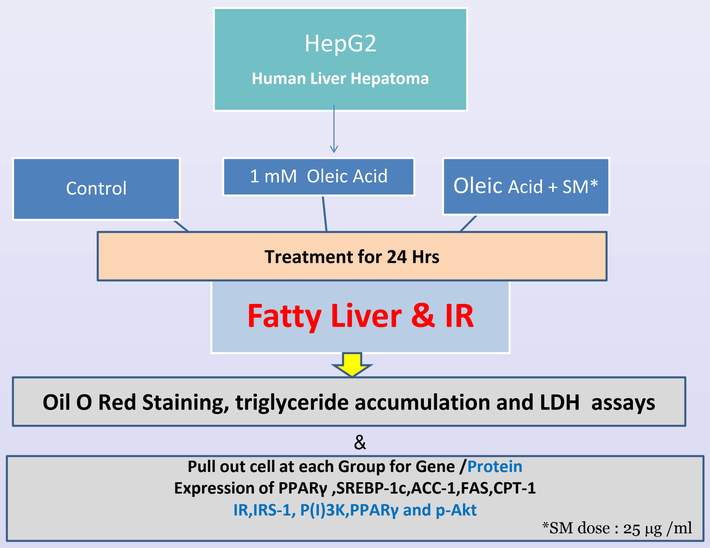
➢Aqueous extract of *Enicostemma littorale* Blume has anti-diabetic activity in the alloxan induced diabetic rats and even in the NIDDM human patients. ***(Maroo J et al., 2002; Vasu V T et al., 2003; Gupta S. et al., 2005., Vasu V T et al., 2005)***

➢Swertiamarin (SM) as a regulator of Hepatic and Adipose tissue gene expression under the control of transcriptional factors like PPARγ, thus confirming that SM improves insulin sensitivity and modulates carbohydrate and fat metabolism. (***Patel et al. 2013)***

➢Our *in vivo* findings suggest potential role of swertiamarin in regulation of transcription control of fat and carbohydrate metabolism in hepatic tissue.

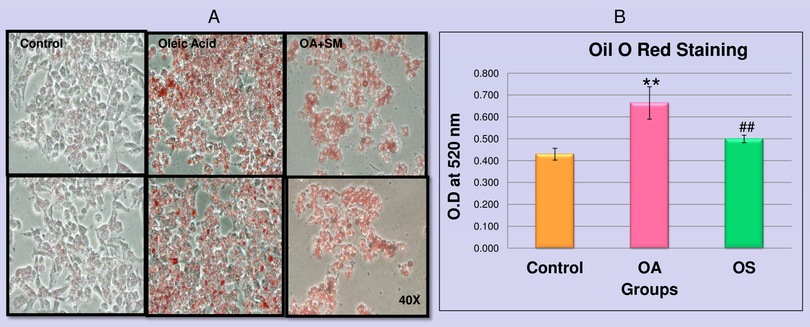
➢Swertiamarin might be effective therapy of non-alcoholic fatty liver disease (NAFLD).

PLAN OF WORK



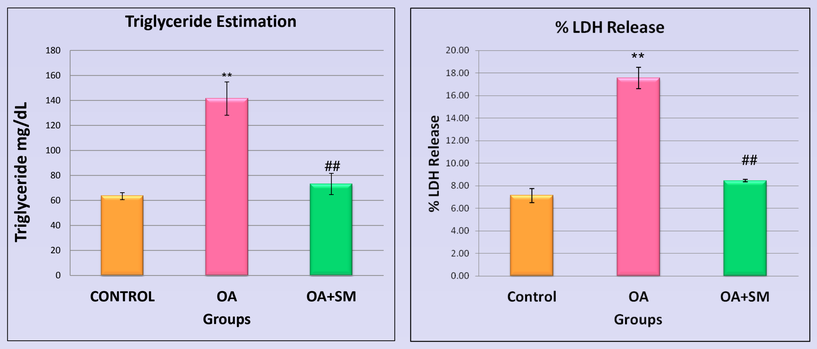
RESULTS

Figure 1: A) OA-induced steatosis in HepG2 cells determined by ORO staining. B) ORO-based colorimetric assay. Quantification of Oil O Red stain after extraction procedure.



p-value **≤ 0.005 as compared to control;
p-value ##≤ 0.005 as compared to OA. n=3

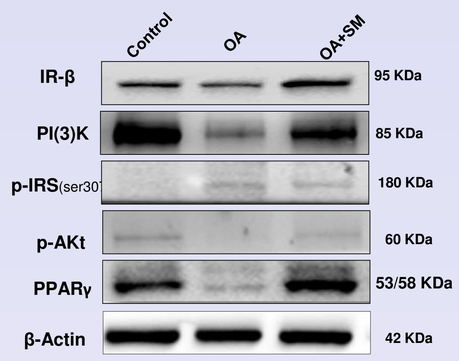
Figure 2A : Effect of swertiamarin on A) Triglyceride accumulation. B) % LDH release.



p-value **≤ 0.005 as compared to control; p-value ##≤ 0.005 as compared to OA. n=3

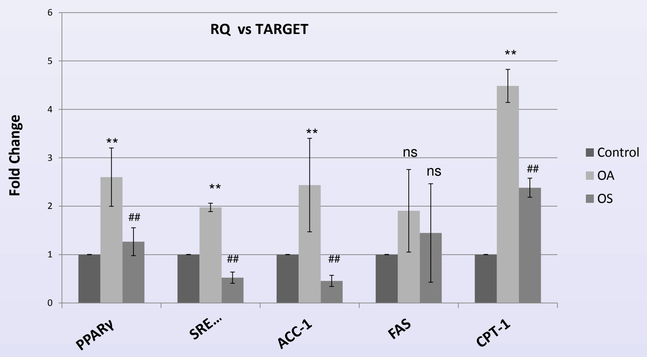
p-value **≤ 0.005 as compared to control; p-value ##≤ 0.005 as compared to OA. n=3

Figure 3 : Effect of Swertiamarin on Signaling pathways using insulin resistant HepG2.



Western blot study showing the effect of SM treatments on the expression of PPARγ and Insulin signaling proteins: IR,IRS-1,Akt and PI(3)K in the Adipocyte as compared to OA treated group . β-actin was taken as an internal control.(15 ug protein)

Figure 4 : Effect of swertiamarin treatments on the expression of fat metabolism genes in the liver steatosis. The expression levels were checked using quantitative PCR.



Data presented as Mean ± SEM of n=3 .
p-value **≤ 0.005 as compared to control; p-value ##≤ 0.005 as compared to OA. n=3.
p-value ; ns > 0.05 as compared to control & OA

DISCUSSION

❖Hepatic steatosis results from increased fatty acid influx to hepatoctyes, reduced lipid oxidation and decreased VLDL excretion .(Cui et al., 2010)

❖HepG2 cells were supplemented with pathophysiologic levels of oleic acid to mimic the influx of excess FFAs into hepatocytes, giving rise to hepatic steatosis. (Barve et al., 2007)

❖Insulin mediated glucose uptake and proliferation of HepG2 cells were hampered in steatosis due to excess fat accumulation which suggest a link between antioxidant imbalance, insulin resistance and obesity-related complications.

❖The results demonstrated that cells incubated with swertiamarin remarkably decreased the ORO staining, TAG accumulation and the % LDH release.

❖Insulin resistance is a major factor responsible for NAFLD and management of this condition reduces its risk.

❖Swertiamarin improved insulin sensitivity by restoring the level of insulin receptor, Akt phosphorylation, PPAR-γ and PI(3)K proteins as previously proved by Patel et al.,2013 in an in vivo model.

❖Swertiamarin controls fatty acid synthesis by down-regulation of SREBP1c and ACC-1.Mitochondrial fatty acid oxidation was reduced by CPT-1 gene expression control.

❖PPARγ, one of the major players for triglyceride biosynthesis and fat metabolism was also shown to be controlled by swertiamarin (Patel et al.,2013).

CONCLUSION

Swertiamarin effectively reversed NAFLD symptoms by decreasing triglyceride accumulation, fatty acid synthesis, insulin resistance and increasing cellular antioxidants in OA induced hepatic steatosis in HepG2 cells. Hence swertiamarin is promising to carry out more experimental and clinical studies to understand the molecular mechanisms to overcome NAFLD symptoms.

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PARP-1 regulates adipocyte differentiation mediated by TGF-ß Signalling pathway

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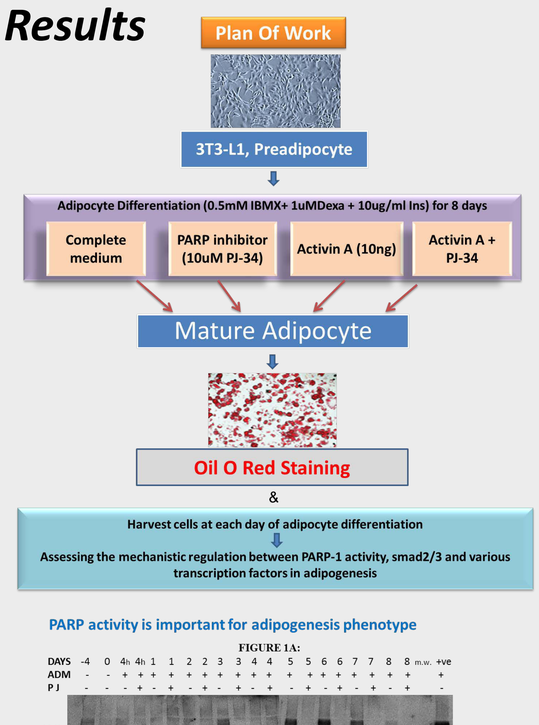
Abstract

Adipose tissue, the storehouse of body fat, plays a key role in regulating glucose and fat homeostasis in the entire body. Hence, being obese with an anomalous accumulation of fat in adipose tissue disturbs their normal functions, which leads to development of type II diabetes and obesity related complications such as hypertension and cardiovascular disorders. White adipose tissue (WAT) mainly governs the storage of energy and its mobilization, and its genesis is the foremost area of interest for researchers envisaging obesity, which is governed by the phenomenon of adipogenesis. Several transcription factors are directly involved in this process among which peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer-binding proteins (C/EBPs) play pivotal roles. There are reports suggesting that TGF-β signalling molecules like Activins, Bone morphogenetic proteins (BMPs) and Smads proteins are involved in this paradigm, although the precise regulatory mechanism for adipogenesis is still elusive. Hence, in the present study 3T3-L1, preadipocytes were used for understanding the role of poly(ADP-ribose) polymerase-1 (PARP-1) in adipogenesis. PARP-1 inhibition and depletion impedes polymer formation and altered smad phosphorylation, which reduces adipogenesis in 3T3-L1, preadipocytes. Exogenously inhibited PARylation of proteins suppresses PPARγ2 transcription by inhibiting PARylation of pSmad2/3 which affects its binding to PPARγ2 promoter. Thus, PARP activity positively regulates adipogenesis, and its inhibition holds promising future possibilities for the treatment of obesity and type II diabetes.

Introduction

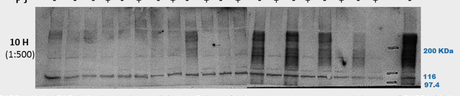
Adipocyte development has emerged as an attractive area of biomedical research in recent years due to the dramatic increase in obesity and obesity related diseases (type-II diabetes, cardiovascular disease and cancers) worldwide. Hyperplasia of the adipocytes is initiated by adipogenesis of the mesenchymal stem cells or preadipocytes. Therefore, understanding the molecular mechanism that regulates the development and growth of adipose tissues is essential to treat and prevent obesity. C/EBP-β and -δ are initial transcription factors involved after the induction of differentiation (Salma et al. 2004). PPAR-γ is a major master regulator of mid-late adipogenesis and in functioning of mature adipocyte (Siersbaek et al. 2012). Poly(ADP-ribose) polymerase-1 (PARP-1) recruitment to the promoters of different PPAR-γ dependent target genes was strongly enhanced upon PAR formation. Binding of PPARγ ligand alters the conformation of PPARγ, resulting into transcriptional activation and subsequent release of corepressors that are regulated by various post translational modifications like poly(ADP-ribosyl)polymerisation and acetylation (Bai et al. 2007). Poly(ADP-ribose)polymerase-1(PARP-1), is an abundant and ubiquitous chromatin associated nuclear protein among 17 PARP family members. PARP-1 catalyzes the nicotinamide adenine dinucleotide(NAD+)-dependent addition of polymer of ADP-ribose(Rayalam et al. 2009) onto a variety of target proteins. PARP-1 activity is necessary for adipocyte differentiation, and increased PARylation can be observed in differentiating 3T3-L1 adipocytes (Erener et al. 2012). PARP-1 regulates adipogensis by controlling histone marks and topoisomerase-2 activity. PARP-2 also inhibits adipogenesis (Bai et al. 2007; Bai and Canto 2012). Members of TGF-β super family, Activin-A/TGF-β ligand inhibits adipogenesis by decreasing PPAR-γ expression. PARP-1 also acts as a Smad-interacting partner. PARP-1 dissociates Smad complexes from DNA by ADP-ribosylating Smad3, which controls the strength and duration of Smad-mediated transcription (Lonn et al. 2010). It has been previously reported that PARP proteins and TGF-β signalling molecules are involved in adipogenesis but the exact mechanism is still not clear. Hence, to understand the role of PARP-1, we targeted PARP-1 mediated interactions with major transcription factors during adipogenesis.

Results



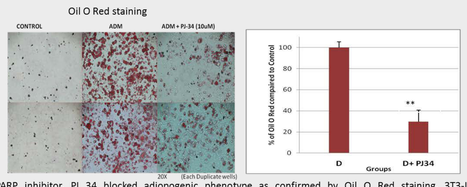
PARP activity is important for adipogenesis phenotype

FIGURE 1A:



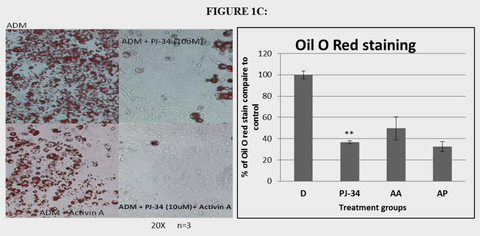
PARP activity (PARylation) was observed during 8 days of adipogenesis in presence and absence of PARP inhibitor PJ-34(10 uM). 20 µg of total protein was loaded in each well and blot was probed with 10H(1:500) antibody.

FIGURE 1B:



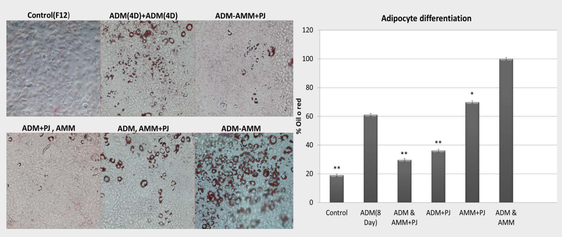
PARP inhibitor, PJ 34 blocked adiopogenic phenotype as confirmed by Oil O Red staining. 3T3-L1 preadipocytes were subjected to adipocyte differentiation using adipogenic cocktail for 8 days and were checked by the Oil O Red staining. (i) Oil O Red staining under 20X magnification under Phase contrast microscope (ii) Quantification of Oil O Red stain after extraction procedure is represented in terms of % of Oil O Red stain compared to control (p-value ** ≤ 0.005, n=3).

FIGURE 1C:



Anti-Adiopogenic effect of PJ-34 combined with TGF-β ligand (Activin A); (i) Oil O Red staining under 20X magnification under Phase contrast microscope (ii) Quantification of Oil O Red stain after extraction procedure is represented in terms of % of Oil O Red stain compared to control. (p-value ** ≤ 0.005)

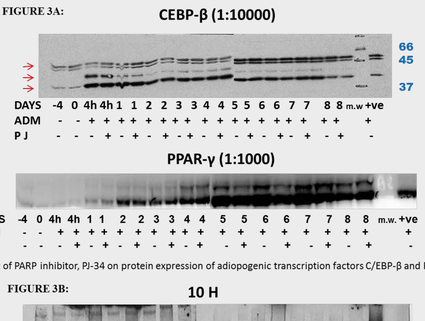
FIGURE 2: **PJ-34 inhibits adipogenesis in early phase of differentiation.**



PJ-34 was treated in early and late phase of adipogenesis, confirmation by (i) Oil O Red staining under 20X magnification under Phase contrast microscope (ii) Quantification of Oil O Red stain after extraction procedure is represented in terms of % of Oil O Red stain compared to control. (p-value ** ≤ 0.005, * ≤0.05) compared to ADM & AMM (VI)

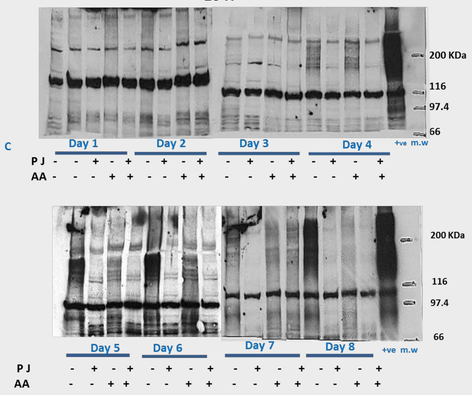
PARylation is mandatory for transcriptional control of adipogenesis.

FIGURE 3A:



Effect of PARP inhibitor, PJ-34 on protein expression of adiopogenic transcription factors C/EBP-β and PPARγ.

FIGURE 3B:



After treatment with Activin-A, PJ-34 and their combinations, PARylation was observed by probing the blot with 10H antibody.

Figure 3C i

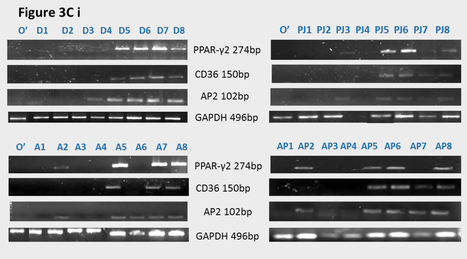
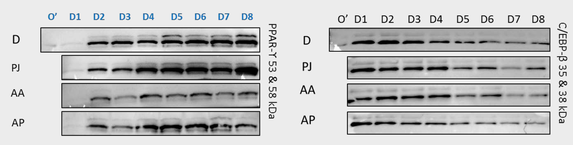


FIGURE 3C ii:

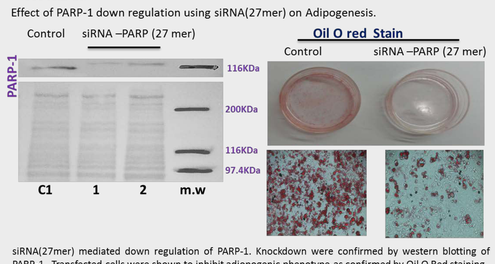


After treatment with Activin-A, PJ-34 and their combinations during adipogenesis (i) PPARγ and C/EBP-β protein expressions were checked by western blotting. (ii) Gene expressions of CD36 and aP2 were checked using semi-quantitative RT-PCR.

Specific role of PARP-1 in adipocyte differentiation.

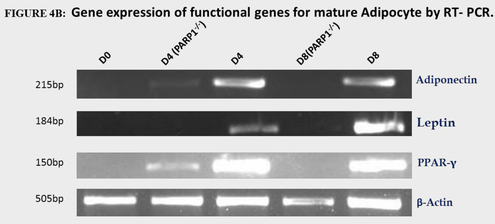
FIGURE 4A:

Effect of PARP-1 down regulation using siRNA(27mer) on Adipogenesis.



siRNA(27mer) mediated down regulation of PARP-1. Knockdown were confirmed by western blotting of PARP-1. Transfected cells were shown to inhibit adiopogenic phenotype as confirmed by Oil O Red staining.

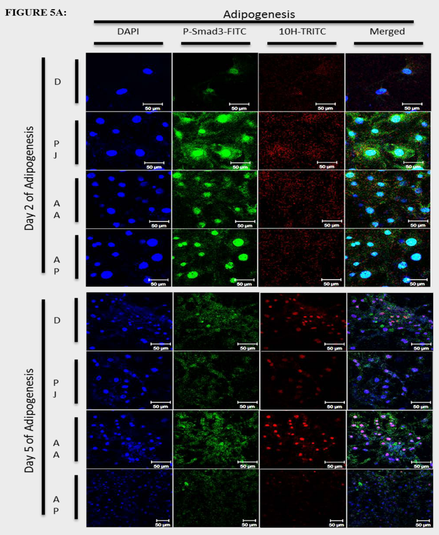
FIGURE 4B: **Gene expression of functional genes for mature Adipocyte by RT- PCR.**



PARP-1 is required for gene expression of adiopogenic transcription factors :PPARγ, Adiponectin and Leptin. The expression levels were checked using semi-quantitative RT-PCR.

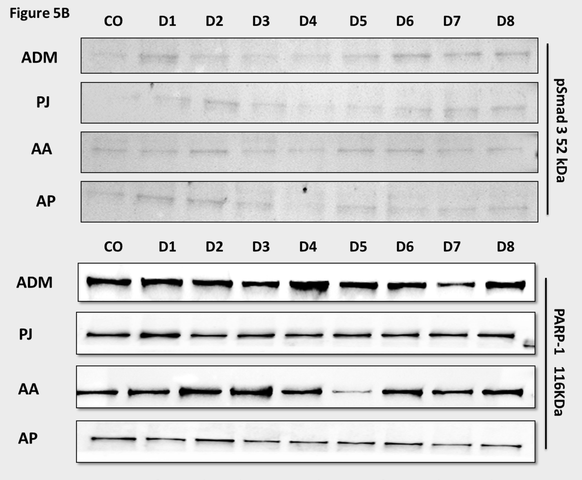
Interaction of PARP-1 and PAR(pADPr) with pSmad3 during adipogenesis.

FIGURE 5A:



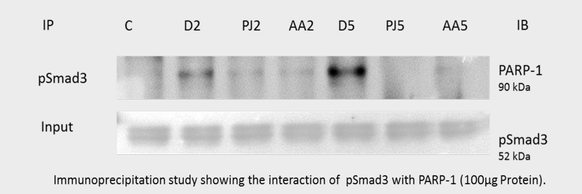
Expression of pSmad3 (tagged with FITC) with PAR (10H tagged with TRITC). ICC were perf ormed on 2nd and 5th day of adipogenesis..

Figure 5B



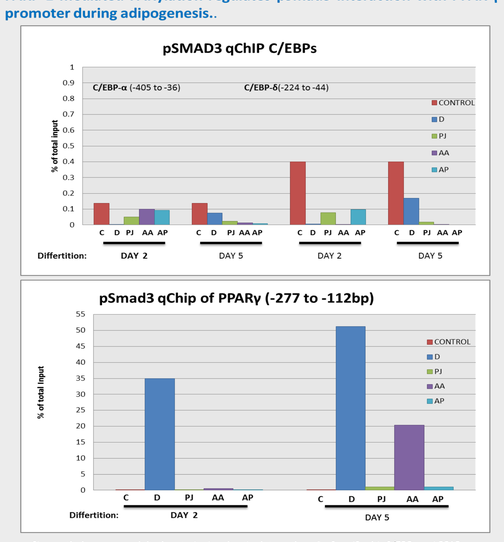
Protein expression of pSmad3 and PARP-1 in chromatin fraction during adipogensis.

FIGURE 5C:



Immunoprecipitation study showing the interaction of pSmad3 with PARP-1 (100µg Protein).

Figure 6 **PARP-1 mediated PARylation regulates pSmad3 interaction with PPAR-γ promoter during adipogenesis..**



Chromatin-immunoprecipitation study showing the interaction of pSmad3 with C/EBPs and PPAR-γ promoters. (250ug of chromatin)

Discussion

- PARP-1 -/- mice showed reduced fat mass (Bai *et al*, 2012), our study proved that PARP-1 activity is necessary for white adipocyte differentiation *in vitro*.
- PARP-1 is found to be positive regulator whereas, TGFβ/ Activin-A are the negative regulators in adipogenesis.
- PARP activity (PARylation) starts at 3rd day and persists till 7th day of adipogenesis *In-vitro*. PARP inhibitor PJ-34 inhibits PARylation as well as adipogenesis more effectively during early phase of differentiation.
- PARylation of PARP-1 is required for its recruitment to the PPAR-γ, which sustains the expression levels of PPAR-γ and its target genes (Erener *et al*, 2012).
- Treatment of PJ-34 during adipogenesis did not alter the expressions of C/EBP-β protein but reduced PPARγ2 protein expression.
- TGF-β regulates adipogenesis by smad3 interacting with C/EBPs, and its transactivation functions effects the transcription of PPAR-γ and leptin promoter (Choy L.,2003).
- PARP-1 interacts with pSmad2/3 in PARylation dependent manner which increases during adipogenesis. ADP-ribosylation of Smad proteins by PARP-1 was identified as a key step in controlling the strength and duration of Smad-mediated transcription (Lonn *et al*, 2010).
- Complex formation between pSmad2/3 and PARP-1 in nucleus leads to PARylation of Smads, which then binds to the target DNA sequence in promoter of C/EBPs and PPARγ2 during differentiation.
- Chromatin immunoprecipitation of pSmad3 and qPCR analysis of C/EBPα/δ and PPAR-γ promoters states that pSmad3 interacts at PPAR-γ2 promoter.

Conclusion

Adipogenesis is inhibited due to reduced polymer formation and binding of Smads. Thus, possible mechanism of action is PARP-1 mediated PARylation regulates Smad2/3 and hence, plays a major role as transcriptional control in adipogenesis. PARP inhibition holds promising possibilities for the treatment of metabolic damage, but for achieving healthy life through PARP based therapies, one will require further understanding of PAR biology, fine-tuning of the dynamics and specificity of PARP inhibitors targeting TGF-β signalling for future use.

Acknowledgement

Firstly, I would like to thank Canadian Commonwealth fellowship that facilitated me to work for six months at Quebec, Canada. Thanks to Dr. Katherine Cianflone for providing 3T3-L1 cells. I would also like to acknowledge DBT-MSUB-ILSPARE Project DBT-India, for Central Instrumentation Facility.

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Presented at **EMBO | EMBL Symposium: 'Translating Diabetes' 30 April - 3 May 2014, Heidelberg, Germany**

Angiotensin-1 converting enzyme Gene polymorphism in Diabetic Nephropathy in West Indian population

MOLMED-2011

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ABSTRACT

Diabetic nephropathy is a clinical syndrome characterized by the occurrence of persistent microalbuminuria in concomitance with insulin- or non–insulin dependent diabetes. Hypertension increases incidence of diabetes which further leads to development of diabetic nephropathy. Angiotensin converting enzyme plays an important role in the regulation of blood pressure. There are many evidences that suggest hypertension plays an important role in the development and progression of Diabetic nephropathy which ultimately leads to end stage renal failure.

The aim of the present study is to access the association of candidate gene polymorphism in the development of Diabetic nephropathy from West India. The Objective is to identify the association of Insertion/Deletion polymorphism in ACE gene. Several studies suggest that there is an association of ACE gene Deletion allele (D) with the progression of Diabetic nephropathy, therefore it is important to look for the genetic association from West India. Present study was carried out with 98 subjects (43 Diabetic nephropathy patients, 12 Diabetes patients, 43 controls.), and we found that frequency of ACE gene Deletion allele (D) is significantly higher in case of Diabetic nephropathy patients as well as Diabetic patients compared to control

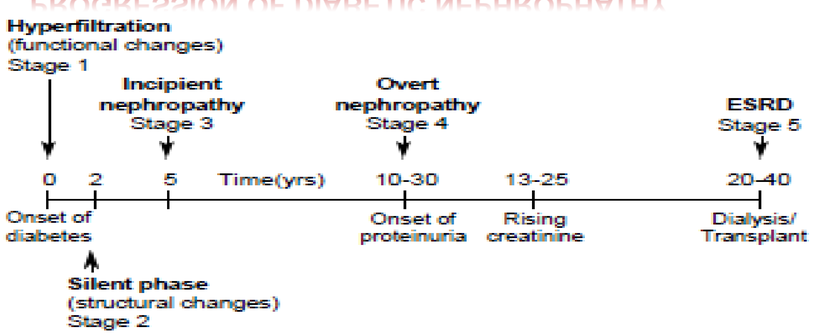
INTRODUCTION

Diabetic nephropathy (nephropatia diabetica), also known as Kimmelstiel-Wilson syndrome and intercapillary glomerulonephritis, is a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli. It is characterized by nephrotic syndrome and diffuse glomerulosclerosis. It is due to long standing diabetes mellitus.

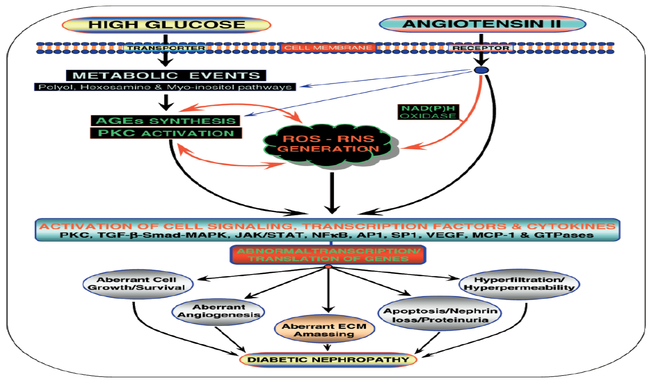
Epidemiology : The syndrome can be seen in patients with chronic diabetes (15 years or more after onset), so patients are usually of older age (between 50 and 70 years old). Several polymorphisms have been reported in genes of the RAS and represent genetic factors that affect both circulating and tissue RASs. These include polymorphisms in the angiotensinogen, ACE, and AGTR1 genes.

There are several SNPs found in the ACE gene which shows association with DN. e.g The rs1800764, rs1799752 and rs9896208 alleles were associated with a risk for development of persistent microalbuminuria (P lower = 0.0009) and severe nephropathy (P=0.006) (Boright et al., 2005),whereas the haplotype analysis showed that (rs1799752, rs4366 and rs12449782) was associated with an increased risk for DN (Hadjadj et al., 2007).

PROGRESSION OF DIABETIC NEPHROPHATHY



$ Subject enrolled in study Group ≥ 50 Years



PLAN OF WORK

Blood collection

⇩

Isolation of genomic DNA

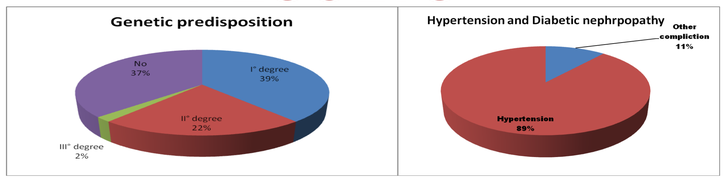
⇩

Amplification of genes by PCR and restriction digestion for accessing the presence of polymorphism.

⇩

Product resolution on DNA PAGE / AGE

RESULTS



Family History:

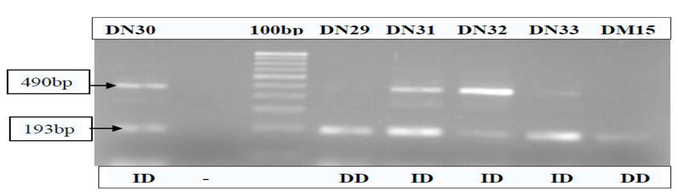
1st° relatives : Father / Mother / Sister / Brother / Daughter / Son

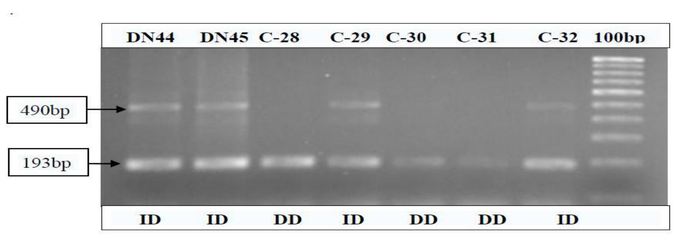
2nd° relatives: Parental grandmother / Parental grandfather / Maternal grandfather / maternal grandmother / Maternal or paternal grand uncles or aunts

3rd° relatives : cousins / nephew / nieces

ACE GENOTYPING

Gene	Position on the chromosome	No. of exons	Locus	Hetero / Homozygous Genotype	Size of Amplicon (bp)
ACE	17q23	27	ACE	II ID DD	490 490 and 190 190





DN : Diabetes Nephropathy & DM: Diabetes Mellitus

	Observed genotype counts (%) #1				Observed allele frequencies		Expected genotype counts @			p value
	II	ID	DD	ID+ DD	I	D	II	ID	DD	* Compare to control
Control (n= 43)	39.53	48.83	11.62	60.45	0.63945	0.36035	17.582	19.8178	5.583	
DN (n= 43)	27.9	48.83	23.25	72.08	0.52321	0.476708	11.771	21.4499	9.771	0.0425*
DM (n=12)	8.333	33.33	58.33	91.66	0.24998	0.74995	0.7498	4.49934	6.749	0.4475

#1 ODD RATIO : 1.68 (0.6838-4.1725) **(95% CI)**

@ Expected Genotype Counts : **Observed vs. expected according to the Hardy–Weinberg equation.**

•Controls vs. patients using the (γ2) chi-square test .

DISCUSSION

➢In our study, 89% of diabetic nephropathy patients were found to have hypertension .

➢ 63% of enrolled diabetic nephropathic patients shows first degree familial history .

➢We have found the frequency of Deletion allele (D) to be higher in diabetic nephropathy patients as compare to control. Our study has found that the frequency of D allele (II+ID) is 72.08% in DN which is significantly higher compare to control (60%) which is similar to findings of Viswanathan v*et al., 2001.*

CONCLUSION

ACE encoding I/D gene polymorphism plays a role in the development of Diabetic nephropathy in west Indian population.

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6. ODD Ratio & (95%, CI) www.hutchon.net/ConfidOR.htm

SYNOPSIS

Synopsis of the thesis on

To Understand the Mechanism of *Enicostemma littorale* Blume Bioactive ingredient in Insulin sensitive Peripheral tissues: *in vitro* and *in vivo* Study.

To be submitted to

The Maharaja Sayajirao University of Baroda

For the degree of

Doctor of Philosophy in Biochemistry

By

Tushar P. Patel, M.Sc.

Under Supervision of
Prof. Sarita Gupta
Molecular Endocrinology and Stem Cell Research Lab

Department of Biochemistry
Faculty of Science
The M. S. University of Baroda
Vadodara- 390 002

From:
Tushar P. Patel
Dept. of Biochemistry,
Faculty of Science,
The M. S. University of Baroda,
Vadodara - 390 002.

16th August 2013

To
The Registrar (Academic Section),
The M. S. University of Baroda,
Vadodara - 390 002.

Subject: Submission of synopsis of the Ph. D. work entitled – "To understand the mechanism of *Enicostemma littorale* Blume bioactive ingredient in insulin sensitive peripheral tissues: *in vitro* and *in vivo* study."

Dear Sir,

Kindly accept the synopsis of my Ph. D work entitled – "**To understand the mechanism of *Enicostemma littorale* Blume bioactive ingredient in insulin sensitive peripheral tissues: *in vitro* and *in vivo* study.**". My date of registration was 27/08/2009 and registration no. is 457.

Thanking you,

Sincerely yours,

(Tushar P. Patel)

(Prof. Sarita Gupta)
Guide

Head, Department of Biochemistry

Dean, Faculty of Science

Introduction

Diabetes mellitus is a multifactorial and complex metabolic disease. It is characterized by alterations in the metabolism of carbohydrate, fat and protein, which are caused by a relative or absolute deficiency of insulin secretion (IDDM) and due to insulin resistance (NIDDM) (Das and Elbein 2006). The pathogenesis of type 2 diabetes mellitus(TIIDM), resulting from defects in insulin receptor (IR) function, IR-signal transduction, glucose transport and phosphorylation, glycogen synthesis, glucose oxidation and dysregulation of fatty acid metabolism contributes to insulin resistance in target tissues and impairment of pancreatic insulin secretion (Saltiel 2001; White 2003). These defects are targets of current pharmacological treatments as well as potential sites for new therapies. In recent years, there has been renewed interest in the treatment of diabetes using herbal drugs, as World Health Organization(WHO) has recommended evaluation of the effectiveness of plants due to side effects of modern drugs (Baby Josheph 2011). There are many treatments available ranging from synthetic drugs like Metformin, Thiazolidinediones, GLP-1, DPP-4 etc. to herbal formulations like *Momordica charantia, Artemisia dracunculus, Gymnema sylvestre,* etc in amelioration of obesity and TIIDM (Cefalu et al. 2008). Large numbers of herbal extracts and their active principal ingredients have demonstrated hypoglycaemic activity for treating diabetes, representing a valuable alternative for control of the disease. However, lack of understanding of mechanism precludes the use of these herbal molecules as a new class of therapeutic agents. *Enicostemma littorale* belonging to family *Gentianaceae* is being used by rural folk for treatment of diabetes. Our lab has well documented, *Enicostemma littorale* Blume owing anti-oxidant, hypolipidemic and anti-diabetic, activities both in animals and human diabetic patients along with amelioration of diabetic complications in various animals models (Jyoti Maroo et al. 2003; Vasu et al. 2005; Bhatt NM et al. 2009; Bhatt NM et al. 2011). Apart from antidiabetic activity islet neogenic property of swertisin and normoglycemia in diabetic mice is also reported (Gupta S 2010; Dadheech et al. 2013). Hence, it can be presumed that the EL extract potentially owes varied beneficiary activities due to the presence of many compounds. Swertiamarin (SM) is the major compound (7% yield) found in EL Blume and its pharmacokinetic study suggested that it rapidly distributed in most of the tissues which was absorbed through liver and eliminated by kidney (Li HL et al. 2011).

There are few reports on role of swertiamarin and various extracts in treatment of TIIDM

and obesity but not much work was carried out at molecular level (Murali et al. 2002; Vaidya et al. 2009; Vishwakarma et al. 2010). Our work focuses on identifying the effect of EL aqueous extracts and swertiamarin, a bioactive ingredient from EL, on genes & proteins expression of insulin sensitive peripheral tissues, like liver, skeletal muscles and adipocytes implicated in carbohydrate & fat metabolism. The major metabolic defects in TIIDM are due to disturbance in insulin signalling leading to insulin resistance. Here, it is important to elucidate the role of swertiamarin in regulation of signaling cascade and transcriptional factors. Poly(ADP-ribose)Polymerase-1(PARP-1) is known to play a crucial role in various cellular functions and differentiation. Hence, we made an attempt to understand the role of PARP-1 in controlling signaling pathways and transcription during adipocyte differentiation which has great impact on TIIDM and Obesity.

Objectives of the study -

1. To assess effect of *Enicostemma littorale* Blume bioactive ingredient in regulation of candidate metabolic gene expression in insulin sensitive peripheral tissue of experimental NIDDM rat model.
2. To study the mechanism of action of swertiamarin: An active lead from *Enicostemma littorale* in insulin resistant peripheral tissue: *in vitro.*
   (a) Oleic acid induced model of Hepatic steatosis HepG2.
   (b) TNF-α induced insulin resistance L6 myocytes.
   (c) Dexamethasone **/** TNF-α induced insulin resistance in adipocyte.
3. Assessment of molecular mechanism of swertiamarin by elucidating it's its anti-adiopogenic potentials.
4. Role of swertiamarin and Poly (ADP) Ribose Polymerase-1 in adipocyte differentiation.

Obj-1: **To assess effect of *Enicostemma littorale* Blume bioactive ingredient in regulation of candidate metabolic gene expression in insulin sensitive peripheral tissue of experimental NIDDM rat model.**

In this study, we attempt to unravel the mechanism of action of swertiamarin by comparing its molecular effects with those of aqueous EL extract in alleviating the insulin resistance in Type II Diabetic Rat model. We investigated hypolipidemic and insulin sensitizing effect in NA-STZ induced experimental non insulin dependent diabetes

mellitus (NIDDM) rats; Swertiamarin(50 mg/kg/day) and aqueous extract(15 grams dried plant equivalent extract/kg/day) were administered to rats orally for 40 days, tight regulation of serum glucose, insulin and lipid profile were observed in both groups. In diabetic rats, swertiamarin enhances insulin sensitivity and glucose metabolism by restoring enzyme activity of G6Pase and HMG-CoA reductase to normal levels and restoring gene expression levels of PEPCK, GK, GLUT-2, PPAR-γ, Leptin, Adiponectin, LPL, SREBP-1c and Glut4. This is the first report that highlights a significant role of SM (*in vivo)* as a regulator of gene expression under the control of transcriptional factors like peroxisome proliferator-activated receptor(PPAR)-γ, thus confirming that SM improves insulin sensitivity and modulates carbohydrate and fat metabolism. Present results strongly suggest that SM can be a potent therapeutic agent for TIIDM (Patel et al. 2013).

Obj-2: **To study mechanism of action of swertiamarin : An active lead from *Enicostemma littorale* in insulin resistant peripheral tissue *in vitro.***

Our Lab previously investigated hypoglycemic, hypolipidemic and antidiabetic effects of EL aqueous extracts in various animal models as well as NIDDM human patients (Vihas T. Vasu et al. 2003; Vasu et al. 2005; Bhatt NM et al. 2009). *In-vivo* study on swertiamarin has also revealed that it has very important role in regulation of hepatic as well as adipose tissue gene expression under diabetic condition (Patel et al. 2013). The present study was undertaken to provide mechanism of swertiamarin in model cell lines, namely HepG2, L6 myocytes and 3T3-L1preadipocytes.

Liver is metabolically active organ. An attempt was made to unravel the mechanism of action of swertiamarin(25µg/ml) in hepatic tissue. Oleic acid(OA)- induced steatosis in HepG2 cell line was confirmed using Oil O Red staining and triglycerides(TG) accumulation assay. Expressions of candidate genes namely GK, PEPCK, GP, Glut2, ACC-1 & FAS in fat and carbohydrate metabolism governed by nuclear transcription factor PPAR-α/γ was investigated.

Presently, the work is focused on TNF-α induced insulin resistance in L6 myocyte, an excellent *in vitro* model for understanding the role of Swertiamarin in regulation of expression of candidate genes in inflammation induced TIIDM condition. L6 myocyte were differentiated into myotubules, which was confirmed using may-grunwald giemsa staining. Insulin resistance was induced by treating these cells for 24 hrs with TNF- α.

Insulin resistant L6 myocytes was than treated with swertiamarin and gene and protein

profiling is under process.

To better understand the effect of swertiamarin, 3T3-L1 mature adipocytes were treated with dexamethasone (Anil Kumar and Marita 2000) to induce insulin resistance which was then evaluated by real time PCR for the expression of major genes involved in fat metabolism. Significant up-regulation of PPARγ, aP2 and CD36 were seen in case of swertiamarin treated dexamethasone induced insulin resistant mature adipocytes. PPARγ is one of the key transcription factor involved in adipocytes gene expression. Increased level of PPARγ leads to increased expression of different genes regulating fat metabolism. Activation of SREBP-1c activates PPARγ that further activates LPL, Adiponectin, ACC-1 and aP2 in the case of swertiamarin treated group. These genes play a major role in fat storage by increasing lipogenesis in insulin resistant adipocytes. The differentiated mature adipocytes were made insulin resistant by dexamethasone/ TNF-α to study the role of swertiamarin (25ug/ml) in insulin signalling cascade by assessing protein level and activity of IR-β, PI(3)K, AKT and PKCs by immunoblotting and immunoprecipitation. Swertiamarin potentiates insulin signalling pathway by improving phosphorylation of insulin receptor, PI(3)K and Akt.

Obj-3: **Assessment of molecular mechanism of swertiamarin by elucidating it's its anti-adiopogenic potentials.**

Adipose tissue, the storehouse of body fat, plays a key role in controlling glucose and fat homeostasis in the entire body. Hence, being obese with an abnormal accumulation of fat in adipose tissue disturbs their normal functions, which leads to development of TIIDM. The differentiation of preadipocytes to adipocytes is divisible into four steps. First, the preadipocytes withdraw itself from the cell cycle and, down regulation of genes responsible for the "preadipocyte phenotype". The second step, is called the "mitotic clonal expansion", which allows a last round of cell division. Then, the cells start to acquire the "early adipocyte phenotype" after 48 hours, followed by "differentiated adipocytes" hence, genes involved in energy storage and fat metabolism such as PPARγ are found to be expressed maximally (Ntambi and Young-Cheul 2000).

The 3T3-L1 preadipocytic cells undergo *in vitro* differentiation, to acquire morphological and biochemical characteristics of adipocytes when treated with IBMX, dexamethasone

and insulin (Holm 2003). Hence, it is most commonly used cell line for assessing *in vitro* anti-obesity potential of any therapeutic agent. Anti-obesity potentials of herbal extracts are also assessed *in vitro* using 3T3-L1 pre-adipocytes as, these cells accumulate TG and release leptin when they differentiate into adipocytes (Rayalam et al. 2009). 3T3-L1 preadipocyte differentiated into these mature adipocytes were confirmed by Oil O red staining as well as triglycerides accumulation. Dose dependent studies(1.56-50ug/ml) were done with swertiamarin for its role in adipocyte differentiation. Various transcriptional factors involved in this process were monitored in time course manner (8 days) for first wave(4-48hrs) transcriptional regulator CCAAT/Enhancer-binding protein β (C/EBP-β) and second wave(after 4Day) PPAR-γ by immunoblotting. AMPK and TGF-β signaling pathways govern adipocyte differentiation, hence in the present study efforts were made to elucidate role in these signal transduction pathways.

Obj-4 : **Role of swertiamarin and Poly (ADP) Ribose Polymerase-1 in adipocyte differentiation.**

PARP-1$^{-/-}$ mice display reduced fat mass deposition and decrease in adipocyte size. (Erener et al. 2012; Li et al. 2012). In addition, adipocyte derived stem cells from PARP-1$^{-/-}$ mice displayed lower expression of PPAR-γ & its target genes upon differentiation (Erener et al. 2012b). It was also suggested that, PARP-1 is mandatory for complete round of DNA replication in adipocyte differentiation (Simbulan-Rosenthal et al. 1996).

Increased PARylation was observed during adipocyte differentiation suggesting role of PARP-1 activity in this process. PARP-1 activity controls PPARγ gene expression allowing sustained expression of PPARγ and its target genes CD36, adiponectin and aP2. Treatment with PARP inhibitor, PJ-34 inhibited polymer formation which was normally induced on 3rd day of adipogenesis and remains till day 7 of adipogenesis. PARP inhibitor treatment did not change C/EBP-β protein expression although activity caused decrease in PPAR-γ protein expression. Swertiamarin and Activin A did not show reduction in polymer formation(PARylation) but are regulating PPAR-γ protein expression. Better efficacy of swertiamarin was observed when the cells were treated with PARP inhibitors. PARP-1 protein level was depleted by antisense (siRNA) technique that showed inhibition of adipocyte differentiation. These studies shows specific role of PARP-1 mediated PAR formation in playing a major role in adipogenesis.

Recent studies also demonstrated that PARP-1 mediates poly ADP-riboslylation which attenuates smads signaling (Lonn et al. 2010). TGF-β/Activin A signaling leads to complex formation of activated smad2/3/4 which further impairs adipogenesis at transcriptional level. TGF-β signaling inhibits adipocyte differentiation by smad3 interacting with C/EBP-β transactivation function (Sheng et al. 2008). In light of these observation we analyzed role of PARP-1 depletion and inhibition of its activity individually, which resulted in dissociation of smads leading to impaired adipogenesis. Further, the interaction between PARP-smad proteins was confirmed by immunofluorescence and immunoprecipitation during adipocyte differentiation. PARP inhibition based therapies thus are promising possibilities in metabolic disorder and TIIDM for future use.

Conclusion : Swertiamarin : An active lead from *Enicostemma littorale* regulates insulin sensitive peripheral tissue gene expression by targeting PPAR-γ & improves insulin sensitivity in TIIDM . Swertiamarin is an effective therapy for the non-alcoholic fatty liver disease (NAFLD) and inflammation induced myocyte *in vitro* insulin resistance condition. Swertiamarin inhibits adipogenesis by controlling PPAR-γ at the transcriptional level. PARP-1 mediated PARylation dissociate smads and hence, plays a major role as transcriptional control in adipogenesis.

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Publications:

1. **Tushar P. Patel**, Sanket Soni, Pankti Parikh, Jeetendra Gosai, Ragitha Chruvattil, and Sarita Gupta, "Swertiamarin: An Active Lead from Enicostemma littorale Regulates Hepatic and Adipose Tissue Gene Expression by Targeting PPAR-γ and Improves Insulin Sensitivity in Experimental NIDDM Rat Model," Evidence-Based Complementary and Alternative Medicine, vol. 2013, Article ID 358673, 11 pages, 2013. doi:10.1155/2013/358673

Poster presented and Abstract published:

1. Poster Presented on the topic **"Effect of the *Enicostemma littorale* Blume on gene expression of candidate metabolic regulators in NIDDM Rat hepatic tissue"** at 2nd Biennial International Conference on New Developments in Drug Discovery from Natural Products and Traditional Medicines at NIPER, November 20-24, 2010
2. Poster Presented on the topic **Angiotensin-1 converting enzyme Gene polymorphism in Diabetic Nephropathy in West Indian population** at Molmed2011- International Conference on Molecular Medicine CHARUSAT-Changa, January 9-11, 2011

Oral presentation:

1. Presented on the topic "**Study of Genetic Susceptibility to Neural Tube Defect and its Association with Maternal Vitamin B12 & Folate Status.**" On 25th Oct 2008 Third Annual Meeting Multi-central Project on Neural Tube Defect at Diabetic Care Unit, KEM Hospital, Pune.

Abstract published :

1. Abstract published on topic **"Evaluation of protective effect of Enicostemma littoral extract against H_2O_2 induced apoptosis in islet of Langerhans" .** On 18th Jan,2013 International conference on "Diabetes and it's complications", CHARUSAT, Anand.

International Conference/Symposium attended :

1. Participated in the International Symposium on **Advance in Molecular Medicine and Clinical Implications,** conducted by Reliance Institute of Life Sciences at Dhirubhai Ambani Life Sciences Centre, Navi Mumbai, on January 24 and 25, 2009.
2. **4th World Congress** IACS "Bridging the gap : **Basic Sciences & Clinical Practice**" organized by The M. S. University of Baroda ,Vadodara, INDIA on 1-3 February, 2011

Achievement:

1. Awarded and successfully completed with **Canadian Commonwealth Fellowship 2011** from November 2011 till April 2012, worked at Dr. Girish Shah Lab, Laval University, Quebec Canada.
2. Awarded DBT-JRF under MSUB-ILSPARE.
3. Awarded University Research fellowship : Nov 2010 to April 2011.

Date: August 16, 2013 **Signature of the candidate**

Tushar P. Patel

(Prof. Sarita Gupta)

Guide

Head **Dean**

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