Novel Anti-Diabetic Formula: Recover Islet β-Cell dysfunction with the implementation of molecular approach

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Abstract

The regulation of PPARα and PPARγ at *m*-RNAs expression level is responsible for Islet β-cell dysfunction and insulin secretion seen in diabetic rats. Many traditional treatments have been recommended in the alternative system of medicine for the treatment of diabetes mellitus. *Stevia rebaudiana* and Gurmmar is a nontoxic natural therapeutic agent revealed to own diuretic, hypotensive and hypoglycemic properties. The ground of this study was to examine the anti-hyperglycemic property, expression of *m*-RNA at PPARα and PPARγ receptors and islet β-cells dysfunction of a combined aqueous extracts of *Stevia rebaudiana* and Gurmmar is on streptozotocin induced diabetic wistar rats. The diabetes induced rats were fed with plant extracts at the increasing dosage of 200mg, 300mg and 500mg of body weight. The combined plant extracts administrated rats revealed a significantly (P<0.001) rise of serum insulin levels and higher reduction in hyperglycemia or hyperlipidemia when compared to the diabetic control rats (P<0.001) and also an increase the *m*-RNAs expansion of PPARα and PPARγ respectively. The histological studies of the endocrine region of pancreas of diabetic rats revealed that shrinkage of β-cells of islets of langerhans. The combined plant extracts treated rats revealed recovery of β-cells. The recovery of β-cells was evident at higher dose level i.e. 500mg/wt extracts fed groups. According to the biochemical, molecular and histological results obtained, it was concluded that the combined plant extracts of this plant may sustain anti-diabetic properties by recovery Islet β-cell dysfunction with the implementation of molecular approach.
Keywords: Stevia rebaudiana, Gurmmar, PPARα and PPARγ

1. Introduction

Diabetes is a leading cause of morbidity and mortality globally, and expected to affect more than 1000 million people by 2030. Conversely, this rising trouble of disease has not been met with an equivalent growth in therapeutic options. The gratitude of the pancreatic β-cell as a central player in the pathogenesis of both type 1 and type 2 diabetes has rehabilitated focus on ways to recover glucose homeostasis by maintaining, humanizing and increasing the function of this key cell type[1]. Type 2 diabetes mellitus results from a combination of insulin resistance and progressive islet dysfunction[2]. In various individuals, β-cell failure may lead the clinical diagnosis of diabetes mellitus, and prospect studies such as the UK Prospective Diabetes Study have shown a continued decrement in

β-cell function despite treatment intervention with sulfonylureas, metformin, and insulin[3]. Thiazolidinediones (TZD) are orally active agents used in the treatment of type 2 diabetes that act as agonists for the nuclear transcription factor peroxisome- proliferator-activated-receptor-γ (PPAR-γ)[4]. However, TZD are characteristically thought to act as peripheral insulin sensitizers, there is rising details from studies of human and animal models that these agents may also act to preserve and/or enhance β-cell function in the setting of progressive type 2 diabetes and insulin resistance[5,6]. PPAR-γ is known to be expressed in the pancreatic islet[7,8], and PPAR-responsive elements have been identified in the promoters of genes involved in glucose-stimulated insulin secretion, including Glut2, Gck,

and Pdx1.[9-13] Information from studies of β-cell lines, humans at risk for type 2 diabetes propose that PPAR-γ agonist function and management guides to protection of β-islet cell mass and rodent models of progressive type 2 diabetes[14-17]. Whereas the studies noted above suggested a direct or indirect effect of PPAR-γ agonists on the biology of the β-islet, no studies to date have observed the molecular or epigenetic mechanisms whereby β-islet cell function is preserved or enhanced in response to PPAR-γ activation. In type 2 diabetes the β-islet dysfunction has been accredited to various etiologies, with glucotoxicity, oxidativestress, lipotoxicity, amyloid, deposition dedifferentiation and endoplasmic reticulum stress [18, 19].

Despite important progress in the management of diabetes using synthetic drugs, many traditional plant treatments are still being used throughout the world. Plants are valued in indigenous systems of medicine for the treatment of various diseases[20]. Medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as dietary supplements to existing therapies[21]. Some of the plants that are being used for the treatment of diabetes have received scientific or medicinal scrutiny and even the World Health Organization's expert committee on diabetes recommends that this area warrants further attention[22]. Almost 85% of the world's population trust on traditional medicines for primary health care and outermost of which rivet the use of plant extracts.[23] In India, roughly 95% of the medicine was plant based in the traditional systems of Ayurveda, Unani and Homeopathy [24].

Recent evidence shows that leaves and shoots from the mulberry tree possess a number of medicinal properties, as well as hypotensive, hypoglycemic and diuretic effects[25]. *Stevia rebaudiana* and Gurmmar leaf extracts were shown to possess hypoglycemic effects in animal models of diabetes mellitus[26]. We recently reported that the extract of *Stevia rebaudiana* and Gurmmar leaves promoted significant hypolipidemic activity in experimental animals[27]. The aim of the present study was to clarify the combined aqueous extracts of *Stevia rebaudiana* and Gurmmar effects on blood glucose, PPARα and PPARγ as well as also determine any possible effect on pancreatic Islet β-cell in the streptozotocin (STZ)-induced diabetic.

To test this hypothesis, we treated 8-week-old Wistar rats feed a high-fat diet (HFD) with oral aqueous extracts of *Stevia rebaudiana* and Gurmmar in the STZ -induced diabetic mice for 4 to 6 weeks. Our results showed that optimize combined aqueous extracts formula of *Stevia rebaudiana* and Gurmmar

treated rats displayed significantly enhanced total-body glucose homeostasis, a ruling attributable at least in part to enhanced insulin secretion and islet β-cell function. We show that these enhancements in islet β-cell function can be explained by an effect of combined aqueous extracts directly maintain Islet β-cell growth and upon regulate glucose-stimulated insulin secretion. Our findings consequently inform a novel model whereby PPARα and PPARγ agonists may exert a direct effect for insulin responsive tissues and for the β-cell to adequate insulin secretion and assurance efficient glucose exclusion, respectively.

2. Materials and Methods

2.1 Plants and Preparation of plant extract: The *Stevia rebaudiana* and Gurmmar leaves were collected from Agriculture University, Jabalpur. The leaves were identified taxonomically by department of Botany, Agriculture University, Jabalpur. The leaves were washed three-times with fresh water, last wash was given by distilled water and air dried in shade at room temperature. The Air dried leaves were grounded into fine powder by an electrical mixture. The powdered leaves were kept in air tight container in deep freezer maintained at 4°C till further use[26,27]. The drug solutions were prepared fresh each time and a known volume of the residual extract was suspended in distilled water and was orally administered to the animals during the experimental period [26].

2.2 Animal treatment procedures: Male wistar rats 8 weeks old, weighing around 180-250g were kept in polypropylene cages (three in each cage) at an ambient temperature of 25±20C and 55-65% relative humidity 12±1 hr light and dark timetable was kept in the animal house till the animals were become accustomed to the laboratory environment, and were fed with commercially available rats foodstuff and had free access to drinking water [26]. All animal experiments were performed in accordance with the NIH guidelines. The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justices Empowerment Government of India and Institutional Animal Ethics Committee guidelines. The approval for the work has been received from the Institution Animal Ethics Committee their letter No. AH/VCP/IAEC/26/14.

2.3 Experimental protocols: The male wistar rats were randomly classified into 5 groups, these groups were identified as follows: Normal group (I), Diabetic (STZ induced) control group (II) rats. Diabetic induced animals were fed with plant extract for 5 days in group (III). The plant extract were administered to animals in increasing dosages of 200mg, 300mg, and 500mg/kg body weight to assess

therapeutic effect of the extracts. Separate groups were maintained for each dose levels. Doses were administered orally for 5 weeks. Exclude normal group all groups rendered diabetic by a single intraperitoneal injection of STZ (CALBIOCHEM-Cat #572201, Lot#B69776) (STZ; 60 mg/kg) freshly prepared in 0.1 mol citrate buffer (pH 6.5). Body weight was recorded at the beginning and end of the experiment in every group. At the end of the experimental period, animals were fasted overnight and autopsied under light ether anesthesia.

2.4 Detection of Biochemical analysis: Blood was drawn from the tail of conscious rats in order to estimate their glucose levels using a glucometer (TRUE result M02085763). Blood glucose, Serum TC, TG, LDL, HDL, and VLD was similarly estimated in pathology lab every week until autopsy from each group.

2.5 Pathological examination of rat pancreas: The male wistar rats were euthanized and pancreases were detached. The common morphologies of the pancreases were studied and documented. Each pancreas was separated into two sections. The one half was used for pathologically examinations to make paraffin-embedded sections and stained by hematoxylin & eosin (H&E). Schmidt criterion was employed for histopathological scoring in pathological evaluation. The other half was subjected to RNA and protein extraction.

2.6 Reverse transcription-polymerase chain reaction (RT-PCR) for PPARα and PPARγ *m*-RNAs expression in pancreatic Islet β-cell: Total RNAs in pancreatic Islet β-cell were extracted by RNA isolation kit (Ambion by life technology Cat #AM1910) and quantified by UV spectrophotometry. A total of 3µg RNA was reverse transcribed into cDNA kit (High-Capacity cDNA Reverse Transcription Kit Cat # 4368814), and the resulting product was used as a template for PCR amplification of PPARα and PPARγ genes. The PCR reaction system was set as 50µl. GAPDH was used as an internal reference under the same experimental conditions. Primers for PPARα, PPARγ and GAPDH were synthesized by Eurofins Scientific, Bangalore. Primers for PPARγ (351bp) were: sense, 5'-CGTGA TGGAAGACCACTCGC-3', antisense, 5'-AACCTG ATGGCATTGTGAGA-3'; Primers for PPARα (435bp)5'-GTTGCAAAGCCTGGGATAG-3', antisense, 5'-GGTAGGCTTCGTGGATTCTC-3' for GAPDH (309bp) was: sense, 5'-AGATCCACAACG GATACATT-3', antisense, 5'-TCCCTCAAGATTG TCAGCAA-3'. The cDNA was subjected to denaturation at 94°C for 2 min, followed by 32 cycles of 94°C for 30 sec, 56°C for 30 sec, and 70°C for 1 min with final extension step at 70°C for 10 min. Samples were electrophoresed on a 1% agarose gel, at 52 volts for two hours, and stained with ethidium bromide (ETBR), and imaged on a E-Gel imager (life-Technology) documentation system. Quantity One Software was used to analyze the relative quantity of each band. The relative amount of PPARα, PPARγ *m*-RNAs was normalized to GAPDH.

2.7 Statistical analysis: The data were analyzed with SPSS 20.0 software. Inter group comparisons were performed with one way ANOVAs. $P<0.05$ was measured as statistically significant.

3. Result and Observation

3.1 Effect on serum insulin, blood glucose and lipid profile analysis: The aqueous extract residue of *Stevia rebaudiana* and Gurmmar were combined (1:1) and administered orally in an aqueous solution at increased dose levels of 200mg, 300mg, and 500mg/kg body wt. to diabetic rats (Table 1). The decreased insulin levels in the diabetic animals were enhanced significantly ($P<0.001$) in the extracts treated animals. The highest increment was recorded at 500mg dose level (83.06%). The aqueous extracts were fed with fasting and diabetes induced rats. The total blood-glucose levels were significantly ($P<0.001$) reduced when compared to the specific control animals. The highest depletion was recorded at 500mg dose level, (64.8%) in fasting rats and (64.3%) in diabetic induced rats. In diabetic control animals (DC) the lipid profile such as; TG, TC, LDL and VLDL levels were significantly increased, whereas HDL levels were decreased when compared to the control rats (Table 1). The aqueous combined extract of both herbs was administered orally at increasing dose levels of 200 mg, 300 mg, and 500 mg/kg body weight, to diabetic rats. The diabetic animals at 200 mg/kg dosage recorded no significant change in the TC, TG, HDL, LDL and VLDL levels. On the other hand when dosage levels were increased to 300 mg and 500 mg/kg body wt., a significant ($P<0.001$) depletion in the total cholesterol level was recorded in the diabetic animals. The depletion in the TC, TG, LDL, and VLDL was dose dependent and the highest reduction in the cholesterol recorded was 22.05%, TG was 27.24%, LDL was 80.24% and VLDL was 26.54% in 500 mg/kg body wt., when compared to the diabetic control animals. The depleted HDL in the diabetic rats increased significantly ($P<0.001$) after the administration of the plant extract. The highest increment was recorded at 500 mg/kg body wt. dosage level (26.54%).

Table: 1: Effect of the aqueous extract of combined plant extract on Serum insulin (µ/ml), Blood glucose (mg/dl), Lipid profile (mg/dl), in STZ induced diabetic animals.

| Parameters	Normal Rats (N)	Diabetic Control Rats (DC)	Experimental groups			*P Values
			200mg/kg Body wt	300mg/kg Body wt	500mg/kg Body wt	
Serum Insulin	18.25± 1.56	11.38±1.12 P<0.001	13.18±1.11 P<0.001	14.26±1.19 P<0.001	15.16±1.16 P<0.001	0.001
Blood Glucose						
Fasting Rats (Control Not Induced Diabetes)	78.8±1.21	69.1±1.8 P<0.001	66±2.18 P<0.005	56.2±1.46 P<0.001	51.1±2.11 P<0.001	0.001
Diabetes Induced Rats	79.1±1.26	392±2.68 P<0.0001	283±8.88 P<0.0001	198±4.12 P<0.0001	130±5.58 P<0.0001	0.001
Lipid Profile						
TC	80.1±1.18	86±3.03 P<0.001	84.3±5.16 P<0.001	82.41±2.13 P<0.001	80.49±2.97 P<0.001	0.001
TG	86.03±1.79	99.57±1.45 P<0.001	93.01±4.81 P<0.001	88.11±2.11 P<0.001	86.96±1.02 P<0.001	0.001
HDL	30.99±1.89	26.44±1.81 P<0.001	27.23±2.09 P<0.001	28.03±3.03 P<0.001	31.25±1.14 P<0.001	0.001
LDL	33.06±2.10	40.85±2.97 P<0.001	38.1±3.36 P<0.001	35.06±2.18 P<0.001	32.11±2.09 P<0.001	0.001
VLDL	17.11±1.09	24.46±1.80 P<0.001	23.46±1.04 P<0.001	21.01±1.06 P<0.001	18.02±2.25 P<0.001	0.001

3.2 Histopathological observation and immunohistochemistry: To elucidate the preventative effects of combined plant extract with different doses on the STZ induced diabetes the pancreatic β- islet cells were histologically examined. The pancreatic tissues were obtained 90 days after the STZ administration with or without a treatment of the combined plant extract and then subjected to H&E staining. The non-diabetic rats showed a normal pancreas structure (Fig.1A), whereas the pancreas of the diabetic control (STZ induced diabetes) showed degenerative and necrotic changes as well as islet shrinkage (Fig.1B) this damage of the β-cells due to STZ induction. On the other hand, studies on the supplementation of combined plant extracts the diabetic rats revealed restoration of size of the islets along with β-cells repair. This recovery of the β-cells was recorded as dose dependant that is form 200mg to 300mg/kg body weight of the combined plant extract (Fig.1C&D) given animals. The plant extract fed animals revealed better restored β-cells of pancreas from the STZ induced damage. The restoration of β-cells was evident at higher dose level of 500mg/by wt extract fed groups (Fig. 1E).

Fig.1. Histopathologic combined aqueous extracts effects of *Stevia rebaudiana* and Gurmmar leaf extract on the pancreas of diabetic rats.

[A]   **[B]**
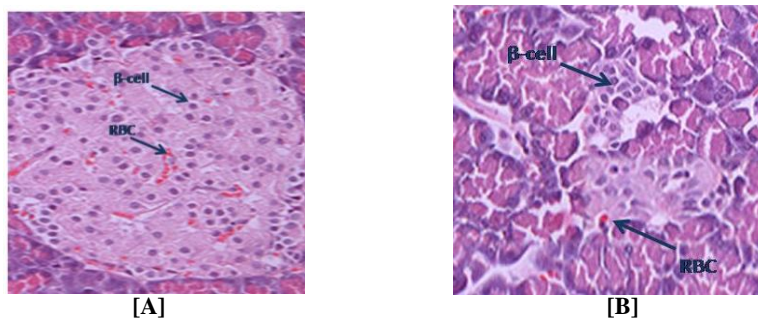
A. The Pancreatic islets of langerhans of normal rat showing RBC cells and β-cells.
B. STZ induced diabetic damaged pancreatic islets showing reduced size and increased damaged β-cells

[C]   **[D]**   **[E]**
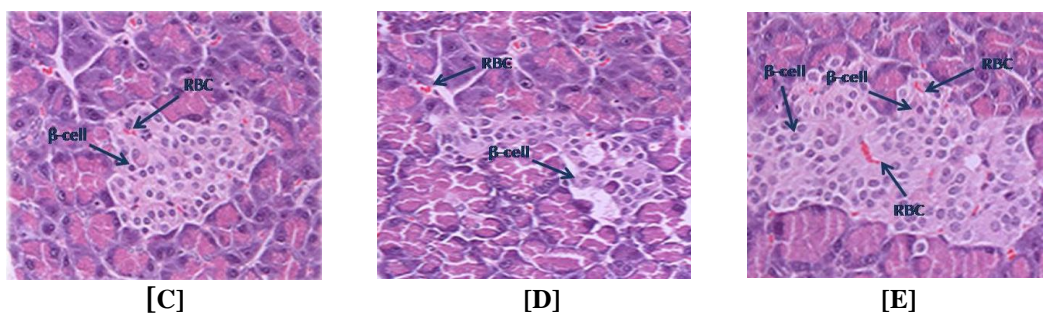
C. Combined plant extract (200mg/kg) treated pancreatic islets show partial revealed better restoration, when compared to the STZ induced diabetic control rats.
D. Combined plant extract (350mg/kg) treated pancreatic islets show partial revealed better restoration, when compared to the STZ induced diabetic and also 300 mg/kg treated rats.
E. Combined plant extract (500mg/kg) treated pancreatic islets shows partial proliferation of β-cells.

3.3 Expressions of the *m*-RNAs for PPARα and PPARγ: Using RT-PCR and competitive PCR method on the total RNA were isolated from the aortae of age-matched controls, untreated diabetic and aqueous combined extract solution at increased dose levels of 200mg, 300mg, and 500mg/kg body white to diabetic rats. Each total RNA preparation (3µg) was reverse-transcribed and half of the cDNA product was PCR amplified using the appropriate primers, 32 cycles being employed. A portion of the PCR reaction product was electrophoresed on a 1.0% agarose gel containing ethidium bromide. We found that the expression of PPARα and PPARγ *m*-RNAs was lower in STZ induced diabetic rat than in normal rat (Fig.2). The lowered PPARγ and PPARα level was modestly increased in 200mg (Fig.3) treated diabetic rats but significantly increased in 300mg treated diabetic mice (Fig.4). The expression of the *m*-RNAs for PPARγ and PPARα was markedly and significantly higher orally administration of 500mg dose (Fig.5) than in 200mg and 300mg treated diabetic rats.

Fig.2. Expression of PPAR α/γ and GAPDH of Normal and STZ Diabetic Induce, Lane M-Marker
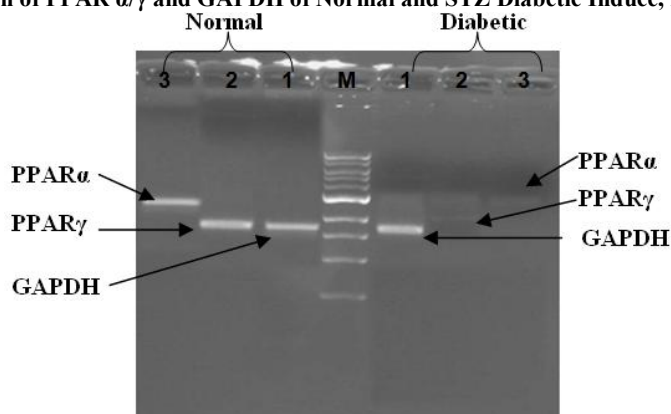

Fig.3. Combined plant extract (200mg/kg) treated expression of PPAR α/γ and GAPDH of STZ Diabetic Induce, Lane M-Marker
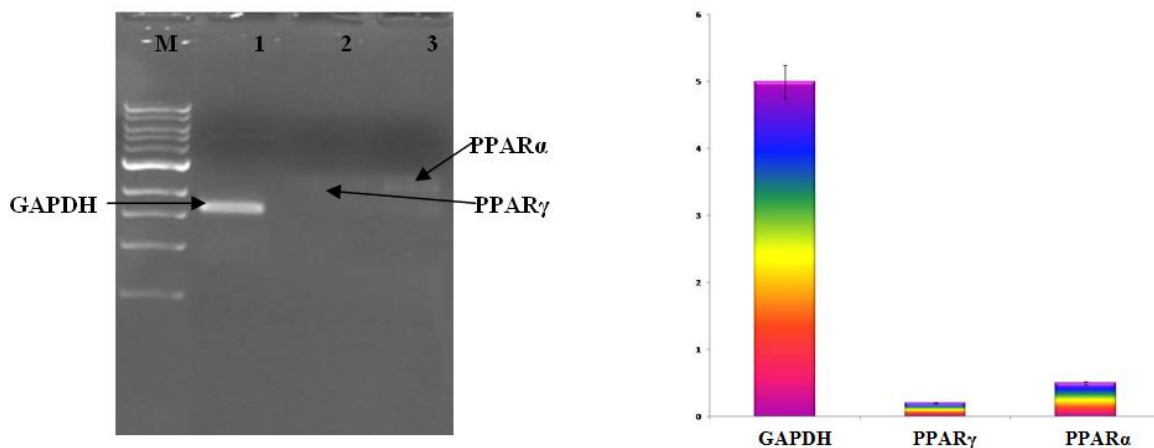

Fig.4. Combined plant extract (300mg/kg) treated expression of PPAR α/γ and GAPDH of STZ Diabetic Induce, Lane M-Marker
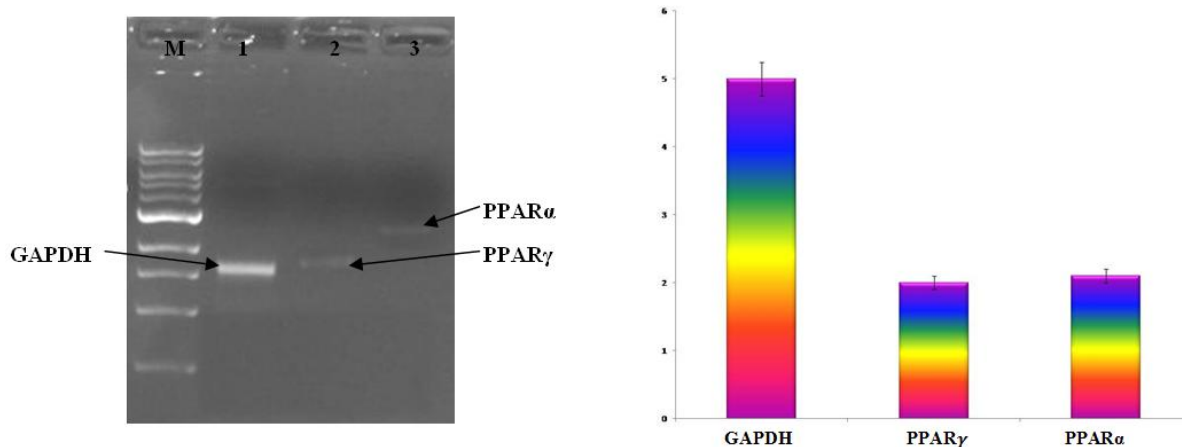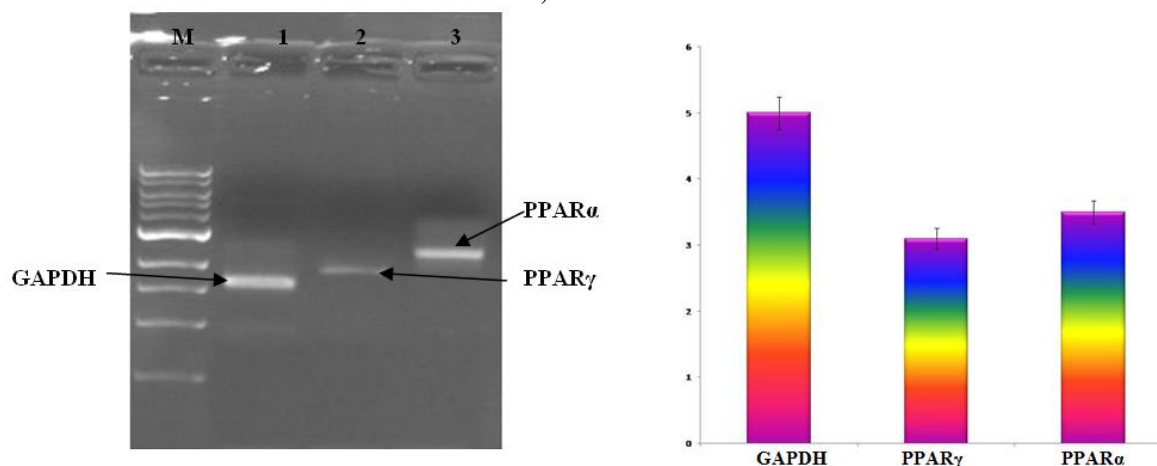
Fig.5. Combined plant extract (500mg/kg) treated expression of PPAR α/γ and GAPDH of STZ   Diabetic Induce, Lane M-Marker

4. Dissuasion

Diabetes mellitus a multifaceted syndrome it is element by insulin resistance or deficiency. That is mostly results from the insufficient transportation of glucose from the vasculature into fat and muscle that finally leads to hyperglycaemia. Diabetic patients reveal distorted glucose fat along with protein metabolism. This dysfunction considerably changes the cellular microenvironment in various different tissue types, leading to "diabetic complications" including microvascular complications such as diabetic nephropathy, neuropathy and retinopathy, along with macrovascular complications including accelerated atherosclerosis causing ischaemic heart and cerebrovascular disease[28]. Diabetes affects both glucose and lipid metabolism.[29] In the post prandial state elevated serum insulin increases lipoprotein lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism[28]. The deficiency of insulin reduces the activity level of lipoprotein lipase, therefore leading to unbalanced lipoprotein metabolism during diabetes[30].

The reduction in the serum insulin levels in the STZ treated rats might be attributed to the reduced secretion of the hormone which might be due to the damage of the beta cells of endocrine pancreas. The STZ selectively destroys the pancreatic cells and induce hyperglycemia[31,32]. Similar studies were recorded earlier in the STZ treated rats, the levels of serum insulin significantly reduced[33]. Nitric oxide has been demonstrated to participate in the β-cell damage during STZ induced diabetes[34].

Indeed, there have been a few reports of functional β-cells regeneration in this high-dose model, despite of limited increases in the numbers of insulin-positive cells shortly after high-dose STZ treatment[27,35,36]. Previous reports have demonstrated that several TCM (traditional Chinese medicine) extracts might exercise antidiabetic effects. Amongst them, mangiferin was accounted to slow fasting plasma glucose level significantly at diverse time periods, and explained additional antidiabetic activities in STZ-diabetic rats[37].

The lipoprotein levels in the alloxan induced diabetic rats in the present study reveal a significant deranged lipoprotein profile. The serum total cholesterol content was increased significantly in diabetic animals. The elevated triglycerides in diabetic animals might be due to the consequence of increased synthesis of triglyceride rich lipoprotein particles VLDL in liver and diminished catabolism[38]. Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver[38-41]. The increased levels of LDL and VLDL in the diabetic animals might be due to over production of LDL and VLDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx[39].

The HDL was significantly reduced in the diabetic rats, which indicate a positive risk factor for atherosclerosis [40]. The levels of serum TC, TG, LDL, and VLDL were significantly reduced in the plant extracts treated diabetic rats. This could be by causes of the reduced hepatic triglyceride synthesis and or reduced lipolysis that might be due to the increase in serum insulin levels in the plant extract treated animals. The HDL was significantly (P<0.001) increased in the plant extract treated animals indicating a reversed atherogenic risk.

The previous occurs when the insulin-producing β-cells in the pancreas are shattered, characteristically during an autoimmune disease[42,43]. The previous is caused by a resistance to insulin jointed among a failure to produce sufficient insulin[44,45]. Both type 1 and type 2

diabetes would finally join to ordinary symptoms for example glucose intolerance and hyperglycaemia. The hyperglycemia in both type 1 and type 2 diabetes mostly results from the loss of β-cell mass that would lead to considerable morbidity in the globe population. Lately, an option and promising approach in expansion is to stimulate the regeneration of endogenous β-cells to replace the deficit in β-cell mass in diabetic patients[45-48]. Peroxisome proliferator-activated receptor a (PPARα and PPARγ), which is activated by specific agonists as fatty acid and fibrates forms heterodimers associated with PPAR response elements in the promoter region of target genes and retinoid X receptor [49]. PPARα and PPARγ play an important role in the liver by regulating the metabolism of lipoproteins and fatty acids[50]. In addition, PPARα is widely expressed throughout the cardiovascular system in the heart, blood vessels (endothelial cells and smooth muscle cells) and monocyte/macrophage cells. The high-dose STZ-induced diabetes mice have been comprehensively used to demonstrate the function of transplanted islets[51,52]. Those studies were based on the assumption that mice treated with high-dose STZ are unable to recover endogenous β-cells function and PPARα and PPARγ activity.

Treatment of type 2 diabetes depends on oral hypoglycemic drugs that contain PPARγ and TZD, among which metformin is the most common. These drugs act on the improvement of hyperinsulinemia, an integral part in the development of type 2 diabetes[53]. Metformin is widely used as a first line of treatment for patients by type 2 DM. Metformin recovers glucose metabolism mostly through suppressing hepatic glucose production[54], but its exact mechanism of action remains unclear. Several reports have concluded that metformin does not significantly affect plasma adiponectin levels[55].

For the first time, our study reported here that the oral administration of combined aqueous extract of *Stevia rebaudiana* and Gurmmar solution at increased dose levels of 200mg, 300mg, and 500mg/kg express the hypoglycemic as well weight reduction activities. It was reported that down-regulations of PPARα and PPARγ may lead to an increased expression of *m*-RNA and this increment may trigger endothelial islet β-cells structure dysfunction in STZ induce diabetic mice.

In the present study, we found that the 500mg oral administrations of aqueous extract regulating the serum insulin, blood glucose and lipid profile, which control the report of hypoglycemic action in STZ diabetic mice and that the expression level was modestly but significantly restored by the

oral administration of 500mg/kg. Current pharmacologic approaches are unsatisfactory in improving such consequences of insulin resistance as diabetic dyslipidemia, abnormal coagulation, fibrinolysis, hyperglycemia and hypertension of which may require the use of at least one medication.

Stevia rebaudiana extract is helpful for hypoglycemia and diabetes because it nourishes the pancreas and thereby helps to restore normal pancreatic function in semi-controlled clinical reports [56]. We found that oral administration of *Stevia rebaudiana* and Gurmmar combined extract, which normalize the blood glucose levels and maintain the lipid profile in STZ induce diabetic mice, which control the report of hypoglycemic actions respectively. This effect of combined extract may, exerts an improvement effect on the endothelial dysfunction seen in the aorta in mice with established STZ induced diabetes. The expressions of the *m*-RNAs for PPARα and PPARγ were significantly decreased in STZ induced diabetic mice (compared with the controls) and this decrease was restored partially, but not completely, by the oral administration of Stevia rebaudiana. We had also observed significant restoration in the islet β-cells dysfunction structure in SR treated diabetic mice respectively.

After the administration of the combined aqueous extract to the STZ induced diabetic rats revealed augmented serum insulin levels. The increment of serum insulin levels might be due to increased secretion of the hormone, which might reflect the probable 'repair' of the damaged beta cells of the endocrine of the pancreas due to STZ. The blood glucose level of combined plant extract fed animal was significantly (P<.001) reduced. The highest depletion was recorded in the 500mg/kg body wt., dosage rats. The levels of serum TC, TG, LDL, and VLDL were found to be significantly reduced in the plant extracts treated diabetic rats. This could be by cause of the reduced hepatic triglyceride synthesis and or reduced lipolysis that may be by causes of the increase in serum insulin levels in the plant extract treated rats. The significantly HDL increased in the plant extract treated rats indicating a reversed atherogenic risk. The histological studies of the endocrine region of pancreas of the diabetic and combined plant extract treated animals revealed that shrinkage of β-cells of islets of langerhans in the diabetic animals. The combined plant extracts treated animals' revealed restoration of β-cells. The restorations of the β-cells in diabetic treated (extract fed) animals corroborate the increased serum binsulin levels in treated animals. The present study suggests

that the combined extract had synergetic hypoglycemic effect revealed by increased serum insulin levels, decreased serum lipid levels and therefore attribute to therapeutic value of the combined plant extracts of *Stevia rebaudiana* and Gurmmar to combat the diabetic condition in rats.

Acknowledgment

References

[1] Amedeo Vetere, Amit Choudhary, Sean M. Burns & Bridget K. Wagner. Targeting the pancreatic β-cell to treat diabetes. *Nature Reviews Drug Discovery*.2014; 13: 278-89.

[2] Prentki, M., and C. J. Nolan. Islet beta cell failure in type 2 diabetes. *J. Clin. Investig*. 2006; 116:1802-12.

[3] U.K. Prospective Diabetes Study Group. U.K. Prospective Diabetes Study 16: Overview of 6 Years' Therapy of Type 2 Diabetes: A Progressive Disease. *Diabetes*1995; 44: 1249-58.

[4] Yki-Jarvinen H. Thiazolidinediones. *N Engl J Med.* 2004; 35:1106-118.

[5] Campbell IW, Mariz S. Beta-cell preservation with thiazolidinediones. *Diabetes Res. Clin. Pract*.2007; 76:163-76.

[6] Gastaldelli A, Ferrannini E, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA. Thiazolidinediones improve beta-cell function in type 2 diabetic patients. *Am. J. Physiol. Endocrinol. Metab*. 2007; 292:E871-83.

[7] Dubois M, Pattou F, Kerr-Conte J, Gmyr V, Vandewalle B, Desreumaux P, *et al*. Expression of peroxisome proliferator-activated receptor gamma (PPARgamma) in normal human pancreatic islet cells. *Diabetologia* 2000; 43:1165-69.

[8] Rosen ED, Kulkarni RN, Sarraf P, Ozcan U, Okada T, Hsu CH, *et al*. Targeted elimination of peroxisome proliferator-activated receptor gamma in beta cells leads to abnormalities in islet mass without compromising glucose homeostasis. *Mol. Cell. Biol*. 2003; 23:7222-9.

[9] Gupta D, Jetton TL, Mortensen RM, Duan SZ, Peshavaria M, Leahy JL. In vivo and in vitro studies of a functional peroxisome proliferator-activated receptor gamma response element in the mouse pdx-1 promoter. *J. Biol. Chem*. 2008; 283: 32462-70.

[10] Im SS, Kim JW, Kim TH, Song XL, Kim SY, Kim HI, *et al*. Identification and characterization of peroxisome proliferator response element in the mouse GLUT2 promoter. *Exp. Mol. Med* 2005; 37:101-10.

[11] Kim HI, Ahn YH. 2004. Role of peroxisome proliferator-activated receptor-gamma in the glucose-sensing apparatus of liver and beta-cells. *Diabetes*. 2004; 53: S60-5.

[12] Kim HI, Kim JW, Kim SH, Cha JY, Kim KS, Ahn YH. Identification and functional characterization of the peroxisomal proliferator response element in rat GLUT2 promoter. *Diabetes* 2000; 49:1517-24.

[13] Moibi JA, Gupta D, Jetton TL, Peshavaria M, Desai R, Leahy JL. Peroxisome proliferator-activated receptor-γ regulates expression of PDX-1 and NKX6.1 in INS-1 cells. *Diabetes* 2007; 56:88-95.

[14] Finegood DT, McArthur MD, Kojwang D, Thomas MJ, Topp BG, Leonard T *et al*. Beta-cell mass dynamics in Zucker diabetic fatty rats. Rosiglitazone prevents the rise in net cell death. *Diabetes* 2001; 50:1021-9.

[15] Gerstein HC, Yusuf S, Bosch J, Pogue J, Sheridan P, Dinccag N, *et al*. Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial. *Lancet* 2006; 368:1096-105.

[16] Yajima K, Hirose H, Fujita H, Seto Y, Fujita H, Ukeda K, *et al*. Combination therapy with PPARγ and PPARα agonists increases glucose-stimulated insulin secretion in db/db mice. *Am. J. Physiol. Endocrinol. Metab*. 2003; 284:E966-71.

[17] Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J, Ochoa C, *et al*. Effect of pioglitazone on pancreatic beta-cell function and diabetes risk in Hispanic women with prior gestational diabetes. *Diabetes* 2006; 55:517-22.

[18] Eizirik DL, Cardozo AK, Cnop M. The role for endoplasmic reticulum stress in diabetes mellitus. *Endocr. Rev* 2008; 29:42-61.

[19] Prentki M., C. J. Nolan. Islet beta cell failure in type 2 diabetes. *J. Clin. Investig* 2006; 116:1802-12.

[20] Arise RO, Malomo SO, Adebayo JO *et al*. Effects of aqueous extract of Eucalyptus globulus on lipid peroxidation and selected enzymes of rat liver. *J Med Plant Res* 2009; 3: 77-81.

[21] Bailey LJ, Day C. Traditional plant medicine as treatment for diabetes. *Diab Care* 1989; 12: 553-64.

[22] World Health Organization. WHO Expert Committee on Diabetes Mellitus, Technical reports Series. World Health Organization. Geneva. 1980; 646:1-80.

[23] Sandhya B, Thomas S, Isabel W, Shenbagarathai R. *African journal of Traditional, Complementary and Alternative Medicines*, 2006; 3:101-14.

[24] Satyavati, G.V., Gupta, A.K., Tandon, N. Medicinal Plants of India, Indian Council of Medical Research, New Delhi. 1987; 2:1-31.

[25] Chen F, Nakashima N, Kimura I, Kimura M. Hypoglycemic activity and mechanisms of extracts from mulberry leaves (foliummori) and cortex mori radicis in streptozotocin-induced diabetic mice. *Yakugaku Zasshi* 1995; 115: 476-82.

[26] Kosta Susmit, Tiwari Archana. Restore islet β-cells dysfunction and study the expression of PPARα and PPARγ in *Stevia rebaudiana* treated mice. *International Journal of Scientific & Engineering Research* 2011; 2: 1-7.

[27] Kosta Susmit, Dangi CBS, Kaur Manpreet, Malviya SN. Determination of Stevioside-

Rebaudioside-a & Gymnemic-Gymnemasins-a in *Stevia rebaudiana* & Gymnema sylvestre Leaves via TLC and Preparative HPLC Medical Science. 2014; 7: 96-101.

[28] Chappell JH Jr, Wang XD, Loeken MR. Diabetes and apoptosis: Neural crest cells and neural tube. *Apoptosis* 2009; 14: 1472-83.

[29] Kim YC, Kim SY, Mellado-Gil JM, Yadav H, Neidermyer W, Kamaraju AK, *et al*. RB regulates pancreas development by stabilizing Pdx1. *EMBO J.* 2011; 30:1563-76.

[30] Freinkel N. Banting Lecture 1980: Of pregnancy and progeny. Diabetes 1980; 29: 1023-35.

[31] Gilman AG, Rall TW, Nies AS, Taylor P. The pharmacological basis of therapeutics. New York: Pergamon. 1990; 8: 1811.

[32] Kurup S., Bhonde RR. Combined effect of nicotinamide and streptozotocin on diabetic status in partially pancreatictomized adult BALB/C mice. *Horm Metab Res* 2000; 32:330-4.

[33] Yoon JW, Ray UR. Perspectives on the role of viruses in insulin dependent diabetes. Diabetes care. 1985; 1:39-44.

[34] Duran-Reyes G, Pascoe-Lira D, Vilar-Rojas C, Medina-Navarro R, Diaz-Flores M, Ortega-Camarillo C, *et al*. Diabetogenic effect of STZ diminishes with loss of Nitric oxide role of ultra violet and carboxy PT10. *Pharmacology* 2004; 71; 17-24.

[35] Fernandes A, King LC, Guz Y, Stein R, Wright CV, Teitelman G. Differentiation of new insulin-producing cells is induced by injury in adult pancreatic islets. *Endocrinology* 1997; 138:1750-62.

[36] Teitelman G, Guz Y, Ivkovic S, Ehrlich M. Islet injury induces neurotrophin expression in pancreatic cells and reactive gliosis of peri-islet Schwann cells. *J Neurobiol*.1998; 34:304-18.

[37] Wang HL, Li CY, Zhang B, Liu YD, Lu BM, Shi Z, et.al. Mangiferin Facilitates Islet Regeneration and β-Cell Proliferation through Upregulation of Cell Cycle and β-Cell Regeneration Regulators. *Int J Mol Sci*. May 2014; 15: 9016-35.

[38] Ginsberg HN. Lipoprotein physiology in nondiabetic and diabetic states. Relationship to atherogenesis. *Diabetes care.* 1991; 14: 839-55.

a. Coppack SW, Jenson MD, Miles JM. *In vivo* regulation of lipolysis in human. *J. Lipid Res*.1994; 35:177-93.

[39] Ohno T, Horio F, Tanaka S, Terada M, Namikawa T, kitoh J. Fatty liver and hyperlipidemia in IDDM of streptozotocin treated shrews. *Life Sci*. 2000; 66:125-131.

[40] KN Bopanna, J Kannan, Gangil Sushma, Balaraman R, Rathod S.P. Antidiabetic and anti hyperlipidemic effect of Neem, Lipidemic effect of Neem seed kernel powder on alloxon diabetic rabbits. *Ind. J. Pharmacology* 1997; 29:162-67.

[41] Freinkel N. Banting Lecture. Of pregnancy and progeny. *Diabetes* 1980; 29:1023-35.

[42] Rossini AA., Mordes JP., Like A.A. Immunology of insulin-dependent diabetes mellitus. *Annu. Rev. Immunol.* 1985; 3:289-20.

[43] Kahn S.E. The importance of β-cell failure in the development and progression of type 2 diabetes. *J. Clin. Endocrinol. Metab.* 2001; 86:4047-58.

[44] Kahn B.B., Flier J.S. Obesity and insulin resistance. *J. Clin. Investig*. 2000; 106:473-81.

[45] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; 414:813-20.

[46] Nishikawa T., Edelstein D., Du X.L., Yamagishi S.I., Matsumura T., Kaneda Y., *et al*. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000; 404:787-90.

[47] Mehta JL., Rasouli N., Sinha A.K., Molavi B. Oxidative stress in diabetes: A mechanistic overview of its effects on atherogenesis and myocardial dysfunction. *Int. J. Biochem. Cell Biol* 2006; 38:794-803.

[48] Kanie N, Matsumoto T, Kobayashi T, Kamata K. Relationship between peroxisome proliferator-activated receptors (PPARα and PPARγ) and endothelium-dependent relaxation in streptozotocin-induced diabetic rats. *British Journal of Pharmacology* 2003; 140: 23-32.

[49] Inoue I, Noji S, Awata T, Takahashi K, Nakajima T, Sonoda M, *et al*. Bezafibrate has an antioxidant effect: peroxisome proliferator-activated receptor a is associated with Cu2+, Zn2+-superoxide dismutase in the liver. *Life Sci.* 1998; 63:135-44.

[50] Yin D, Tao J, Lee DD, Shen J, Hara M, Lopez J, *et al*. Recovery of islet beta-cell function in streptozotocin- induced diabetic mice: an indirect role for the spleen. *Diabetes* 2006; 55:3256-3263.

[51] Ar Rajab A, Dawidson IJ, Harris RB, Sentementes JT. Immune privilege of the testis for islet xenotransplantation (rat to mouse). *Cell Transplant*.1994; 3:493-98.

[52] Gerich JE. The genetic basis of type 2 diabetes mellitus: Impaired insulin secretion versus impaired insulin sensitivity. *Endocr. Rev.* 1998; 19: 491-03.

[53] Hundal RS, Krssak M, Dufour S, Laurent D, Lebon V, Chandramouli V, *et al*. Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes* 2000; 49: 2063-9.

[54] Tiikkainen M, Häkkinen AM, Korsheninnikova E, Nyman T, Mäkimattila S, Yki-Järvinen H. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance and gene expression in adipose tissue in patients with type 2 diabetes. *Diabetes* 2004; 53: 2169-76.

[55] Jeppesen PB, Gregersen S, Poulsen CR, Hermansen K. Stevioside acts directly on pancreatic beta cells to secrete insulin: actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K+-channel activity. *Metabolism* 2000; 49: 208-14.