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Research Article

Effects of Pterostilbene isolated from *Pterocarpus marsupium* on High Fat Diet induced Diabetic Rats

Bhavna Grover^{1*}, Partha Roy²

¹B.R. Ambedkar Govt. College, Sriganganagar, Rajasthan, India

²Molecular Endocrinology Laboratory, Department of Biotechnology, Indian Institute of Technology Roorkee, Uttarakhand, India

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ABSTRACT

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The present study tested the anti-diabetic and hypolipidemic potential of pterostilbene isolated from *Pterocarpus marsupium* plants on high fat diet-induced diabetic rats. The high-fat diet-fed rats showed increased serum glucose, cholesterol, triglycerides and insulin resistance ($p < 0.05$). However, treating the animals with pterostilbene different biochemical parameters like glucose tolerance, glycogen content, glucose homeostatic enzymes like glucose-6-phosphatase and hexokinase, adipocytes differentiation, and the expression profiles of different genes regulating carbohydrate and lipid metabolism were improved significantly ($p < 0.05$). Further, pterostilbene was found to demonstrate dual regulation activities for peroxisome proliferators-activated receptors (PPAR) (both PPAR α and PPAR γ). In conclusion, these results show that pterostilbene acts as a potent anti-diabetic molecule. Hence further in-depth mechanistic studies are warranted to use this phytochemical as a medicine.

INTRODUCTION

Defects in carbohydrate metabolism and consistent efforts of the physiological systems to correct this imbalance place overexertion on the endocrine system, leading to the deterioration of endocrine control and diabetes. Biomedical science has unraveled complex and nonlinear physiological and pathobiological processes involved in causing/fostering diabetes and therefore warrants the use of the single drug or a combination of drugs targeting multiple sites.^[1-3] Towards this end, the present paper discusses the isolation and biological characterization of a potent bioactive molecule from a common medicinal plant called *Pterocarpus marsupium* Roxb. towards the treatment of diabetes.

P. marsupium Roxb (Sanskrit: Pitasala) (Leguminosae), also known as Indian kino or Bijasar, is traditionally used in Indian folklore medicine to treat diseases like diarrhea,

toothache, fever, urinary tract infections, skin infections and diabetes.^[4-6] Several phytochemicals have been isolated and characterized from *P. marsupium* aqueous extract like pteroside, pteroisoside, marsuposide, marsupin, protosupin, and pterostilbene, sesquiterpene.^[8,9]

Pterostilbene (trans-3, 5-dimethoxy-4'-hydroxy-stilbene), a phytoalexin polyphenolic compound isolated from *P. marsupium* stem heart wood (Fig. 1) is the analogue of resveratrol, a potential molecule. It is being intensively investigated for its multiple health benefits^[6-9] Unlike resveratrol, pterostilbene did not receive much attention despite few preliminary reports showing the anti-diabetic and hypolipidemic potential of this molecule where it has been reported to lower the blood glucose levels significantly.^[8,10] Pterostilbene is also an established agonist for PPAR with special emphasis on PPAR α , a transcription factor involved in lowering cholesterol and other blood lipids.^[11]

*Corresponding Author: Bhavna Grover

Address: B.R. Ambedkar Govt. College, Sriganganagar, Rajasthan, India

Email: dr.bhavna2018@gmail.com

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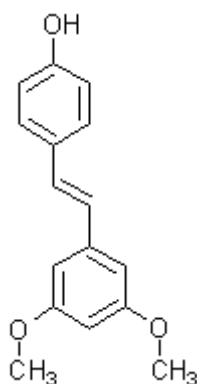


Fig. 1: Structure of Pterostilbene.

Based on previous reports available and results obtained from preliminary studies from our laboratory, we carried out a stepwise activity-based fractionation of aqueous extract using high fat diet diabetic rat models. Finally pterostilbene was purified and identified as the most active constituent in the active fraction of the extract of *P. marsupium* showing potent anti-diabetic, anti-hyperlipidemic, and anti-hyperglycemic activities. Based on results obtained from the present study, it is evident that pterostilbene can combat various targets of insulin resistance and, hence, improve the type II diabetic condition.

MATERIALS AND METHODS

Plant Material

The heart wood of *PM* plant was purchased from a local vender of Roorkee. The plant materials were identified as per the literature of Ayurveda and by local in-charge of herbal garden and also confirmed by Dr. H. S. Dhaliwal, Professor of Plant Biotechnology, Indian Institute of Technology Roorkee.

Purification of Pterostilbene

Pieces of the stem of *Pterocarpus marsupium* were thoroughly washed with water and dried in the shade. Dried stem pieces were ground into a fine powder and soaked overnight in warm water with continuous stirring. After soaking, the mixture was filtered with Whatmann No.1 filter paper. Procedures were repeated until the water stopped changing its color after soaking. The combined filtrate was lyophilized to obtain a concentrated powder.

The powder obtained above was re-dissolved in as little water as possible and washed thrice with chloroform to remove lipids. The residual layer was extracted very with ethyl acetate (EtOAc). The extracts were then pooled together and concentrated using Buchi rotatory evaporator under reduced pressure. Obtained viscous material was chromatographed over a silica gel column using *n*-hexane, EtOAc, and methanol as elutents. All eluted fractions (5 fractions) were freed of solvent, out of which the first fraction showed potent anti-hyperglycemic

effect. This active fraction was identified as pterostilbene by HPLC (Waters, USA) using the commercial standard.

Animal Groups and Treatment

All the experiments were performed as per the Institutional Animal Ethics Committee (registration number: 563/02/a/CPCSEA) and had the prior approval of the project through the same committee. Experiments were carried out on pathogen-free male albino Wistar rats, *Rattus norvegicus*, aged 3–4 weeks. The animals were fed *ad libitum* with a balanced animal pellet diet (Ashirwad Animal Feed Industries, Punjab, India) or High-fat diet for 16 weeks, prepared in house according to DIO (Diet-induced Obesity) Diets, New Brunswick, NJ, USA. The animals had access to normal drinking water at all times.

The animals were randomly divided into four groups (n = 8) as given below:

- Group I - Normal pellet diet-fed (control)
- Group II - High fat diet fed (diabetic)
- Group III - High fat diet fed rats treated with 100 mg/kg pterostilbene (treated).
- Group IV - High fat diet fed rats treated with 20 mg/kg Metformin (metformin)

On completion of 16 weeks, the rats from group III and IV were treated with pterostilbene (dissolved in saline) and metformin, respectively, for another 8 weeks. Rats from groups I and II were gavaged with saline as control.

Effects on Fasting Blood Glucose (FBG) and Glucose Tolerance Test (GTT)

FBG and GTT were determined 24 h after the completion of 8 weeks of treatment of animals. The blood glucose level was measured using GOD-POD glucose estimation kit (Excel Diagnostics Pvt. Ltd., Mumbai, India).

Estimation of Lipid Profile in Blood Samples

Approximately 48 h after completing 8 weeks of treatment, the blood samples were collected from all four groups of animals and lipid profile was determined using commercially available kits (Transasia Bio Medical Limited, Mumbai, India) as described previously.^[12]

Biochemical Estimation of Enzyme Activities and Tissue Glycogen Content

Glucose-6-phosphatase (G6Pase) and hexokinase activities were measured using the slandered method as described previously.^[12] The enzyme activity was expressed as unit per gram tissue.

Glycogen content of liver and skeletal muscles was measured according to the earlier described method using anthrone reagent.^[12]

Serum Insulin

To analyze the effect of pterostilbene on insulin release, serum insulin levels were measured in all the groups using enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (Cayman Chemical, USA).



Semi Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Two-step semi-quantitative RT-PCR method was used to measure gene expression profile in different groups. Primer sequences and optimal PCR annealing temperatures used were as described previously.^[12] The intensity of the bands on gels was converted into digital image with a gel analyzer.

Western Blot Analysis

The tissue extracts were immunoblotted with polyclonal antibodies for PPAR γ and α (kindly donated by Dr. A. Bandyopadhyaya, Indian Institute of Chemical Biology, Kolkata, India).

Histopathological Studies

For histopathological analysis, tissue samples, i.e., pancreas, liver and adipose tissues, were processed as described previously.^[12]

In-vitro Analysis of Anti-diabetic Potential of Pterostilbene

Estimation of Glucose Uptake by Rat Psoas Muscle Tissue

Psoas muscles obtained from high-fat diet diabetic rats were incubated with pterostilbene in the presence and absence of insulin to evaluate the effect of pterostilbene on muscle glucose uptake. After isolation, the muscle tissues were processed and incubated following the method described earlier.^[12]

Peroxisome Proliferators activated Receptor Transactivation Assay

Reporter gene-based assay was performed as per the described method^[12] to check if pterostilbene can activate PPAR.

Adipocyte differentiation assay

The adipocyte differentiation assay was performed as described previously.^[12] Pterostilbene (50 mg/L) was added in the media during assay days. Rosiglitazone was used

as positive control. The differentiation was estimated based on accumulated triglyceride content using the commercially available kit (Transasia Biomedical Limited, Mumbai, India). Oil O Red staining was performed, according the described method.^[14]

Statistical Analysis

Values were expressed as mean $\pm$ S.E.M. The statistical significance was evaluated by one-way ANOVA at 5% level of significance. The statistical package used was Origin 6.1 (Origin Lab Corporation, USA).

RESULTS

Fasting Blood Glucose Level and Oral Glucose Tolerance Test

Fasting blood glucose level of high-fat diet-fed diabetic group showed a significant elevation compared to their control and treated counterparts ($p < 0.05$). However, this level was reduced down (about 40%) upon treatment with 100 mg/kg of pterostilbene (FBG data, Fig. 2). An acute elevation of blood glucose level by 30 minutes followed by a sharp decline at 60 minutes (25%) was seen in treated rats with glucose load during the oral glucose tolerance test (OGTT). Further reduction in blood glucose level was parallel with that of the metformin-treated group albeit in a lesser efficient manner (Fig. 3) ($p < 0.05$).

Effects on Plasma Lipid Profile

High-fat diet-induced diabetes caused hyperlipidemia i.e. high triglycerides (TG) levels accompanied by a raised concentration of low-density lipoprotein (LDL) and diminished high-density lipoprotein (HDL). However, treatment with pterostilbene showed significant improvement in lipid profile as reported by other authors.^[15] As shown in Table 1, about 12 and 48% reduction in TC and TG levels, respectively, were found in diabetic rats treated with pterostilbene ($p < 0.05$). In addition, HDL levels were increased by 14% (as compared to the diabetic control) in pterostilbene treated rats.

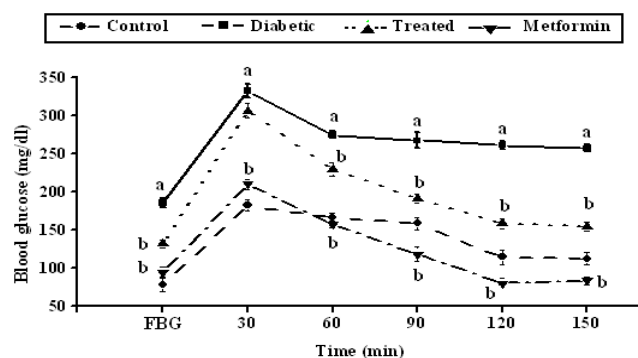


Fig. 2: Oral glucose tolerance test (OGTT) in high fat diet induced diabetic rats in response to pterostilbene. Results are mean $\pm$ S.E.M. of $n = 8$. FBG, Fasting blood glucose. a and b, represents significant level of difference with respect to control and diabetic group, respectively at each corresponding time period ($p < 0.05$).

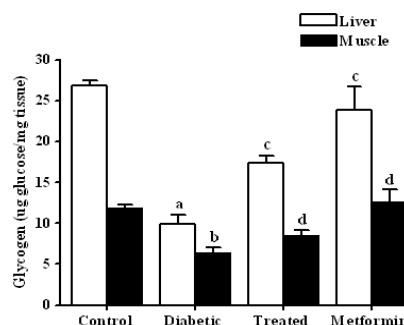


Fig. 3: Effect of pterostilbene on liver and muscle glycogen contents in high fat diet induced diabetic rats. Data are expressed as mean $\pm$ S.E.M; $n = 8$. a and c, indicates the significant level of differences in liver glycogen, b and d, indicates the significant level of differences in muscle glycogen levels, as compared to control and diabetic groups, respectively ($p < 0.05$).

Effects on Hepatic and Muscle Glycogen Content

High fat diet resulted in a severe depletion of liver and muscle glycogen content which was also evident from histological analysis of liver tissues where marked steatosis was seen. Further treatment with pterostilbene caused improvement in glycogen content of liver and muscle by 75 and 17% (Fig. 3).

Enzyme Activity in Liver

Induction of diabetes with a high fat diet resulted in an increased activity of Glucose-6-phosphatase in the diabetic group of animals compared to that of control animals (Fig. 4). However, treatment with pterostilbene resulted in a significant reduction (40%) in its activity which was almost the same as metformin and control groups ($p < 0.05$).

Hexokinase, another important glucose homeostatic enzyme, showed an almost 25% increase in its activity on treatment with pterostilbene as compared to diabetic counterpart (Fig. 4).

Serum Insulin

Feeding the rats with a high fat diet resulted in an elevation of serum insulin level compared to the control diet group (Table 2). On the other hand, treatment of diabetic rats with pterostilbene caused a marked decrease in the elevated serum insulin level in these animals ($p < 0.05$).

Gene Expression Profile

The expression profile of various target genes in liver, muscle, adipose tissue, and kidney was studied in control, diabetic,

and treated animals. As shown in Fig. 5a, the expression of glucokinase was increased in treated rats and at the same time there was a clear reduction in the expression of gluconeogenic enzymes, glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK).

Expression of Glut-4 was significantly low in both muscles and adipose tissues in diabetic group as compared to control group (Fig. 5b). However, treatment with pterostilbene resulted in a profound increase of this gene's expression almost close to metformin albeit at a lesser extent in muscles (Fig. 5b). When tested for aldose reductase, a marker for diabetes-related kidney complications, it was also significantly down-regulated by this phytochemical (Fig. 5b).

Effects of pterostilbene on PPAR α and PPAR γ activities were prominent, as was evident from various experimental parameters. PPAR γ and PPAR α , which regulate carbohydrate and lipid metabolism and control the adipocyte differentiation, showed increased expression after the treatment with pterostilbene in adipose tissues (Fig. 5c). In addition, tumor necrosis factor alpha (TNF α), a multifunctional cytokine, is implicated as a pathogenic factor in the development of insulin resistance because of its multitude of effects on adipose tissue metabolism. In our study, the expression of this cytokine was upregulated in diabetic rats, but treatment with pterostilbene led to a significant decline in its level (Fig. 5c). In way, it was marginally more efficient than metformin in decreasing the expression of TNF α gene.

Table 1: Plasma levels of various lipids after eight weeks of treatment with *pterostilbene* on high fat diet induced diabetic rats

Treatment Group Plasma Lipid level (mg/dL)					
	TC	HDLC	LDLC	VLDLC	TG
Control	154 $\pm$ 5.6	44.1 $\pm$ 1.6	77 $\pm$ 2.8	32.9 $\pm$ 1.2	165 $\pm$ 6.2
Diabetic	220 $\pm$ 7.2 ^a	28.5 $\pm$ 1.9 ^a	112.3 $\pm$ 4.3 ^a	79.6 $\pm$ 1	4a 398 $\pm$ 6.8a
Treated	192 $\pm$ 4.2 ^b	40.7 $\pm$ 1.8 ^b	118 $\pm$ 1.6	41.2 $\pm$ 1.1b	206 $\pm$ 5.6b
Metformin	173.2 $\pm$ 4.7 ^b	32,57 $\pm$ 1.81 ^b	89.5 $\pm$ 1.7 ^b	38.1 $\pm$ 1.2 ^b	190 $\pm$ 5.9 ^b

Values are presented as mean $\pm$ S.E.M., n = 8;

a and b, represent statistically significant ($p < 0.05$) as compared to normal control and diabetic groups respectively among each class of lipids. TC, Total Cholesterol; HDLC, High Density Lipoprotein Cholesterol; LDLC, Low Density Lipoprotein Cholesterol; VLDLC, Very Low Density Lipoprotein Cholesterol; TG, Triglyceride.

Table 2: Serum levels of insulin after eight weeks of treatment with *pterostilbene* on high fat diet induced diabetic rats

Treatment Serum insulin (ng/mL)	
Group	
Control	3.2 $\pm$ 0.13
Diabetic	7.8 $\pm$ 0.11 ^a
Treated	4.6 $\pm$ 0.15 ^b
Metformin	4.1 $\pm$ 0.09 ^b

Values are presented as mean $\pm$ S.E.M., n = 8;

a and b, represent statistically significant ($p < 0.05$) as compared to normal control and diabetic groups respectively.



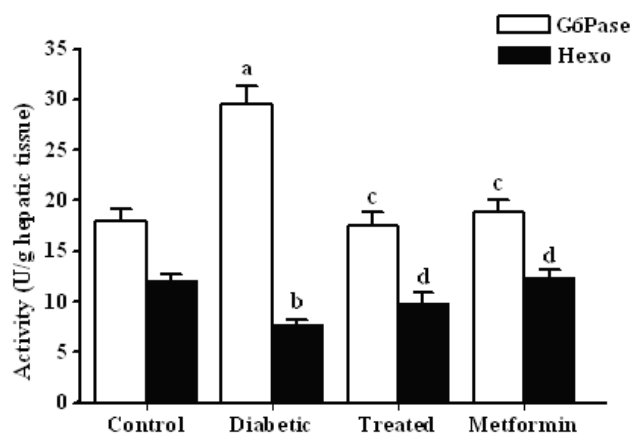


Fig. 4: Effect of pterostilbene on hepatic glucose-6-phosphatase and hexokinase activity in high fat diet induced diabetic rats. Data are expressed as mean $\pm$ S.E.M; n = 8. a and c, indicates the significant level of differences in glucose-6-phosphatase; b and d, indicates the significant level of differences in hexokinase activity levels, as compared to control and diabetic groups respectively ($p < 0.05$). G6Pase, glucose-6-phosphatase.

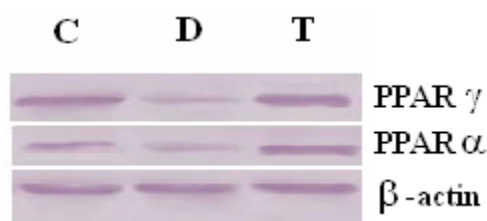


Fig. 6: Immunoblot showing the expression of PPAR α and PPAR γ proteins in adipose tissues from control, diabetic and pterostilbene treated animals. C, control group; D, diabetic group; T, treated group

Immuno Blot Analysis

Changes in the expression profile of PPAR α and PPAR γ was further confirmed by western blot analysis. Immunoblot analysis clearly showed a significant improvement in the expression of both PPAR α and PPAR γ in pterostilbene-treated groups as compared to diabetic counterparts (Fig. 6).

Histopathological Studies

Treatment with pterostilbene for eight weeks resulted in a clear protective effect on the liver and adipose tissue from the treated group compared to the diabetic group. Pterostilbene showed a direct impact on adipose tissue, wherein it stimulated differentiation of new smaller adipocytes along with increased visible adipose tissue depots almost similar to the control group as compared to that of the diabetic group (Fig. 7a to c).

To check if pterostilbene has any toxic effect on the physiological system, histological sections of the liver from a treated group of animals were compared with the control group, which did not show any sign of the toxic effect. One major observation in the diabetic rats was the severe steatosis, i.e. accumulation of lipid droplets within the liver, which was not found in control rats. On the other

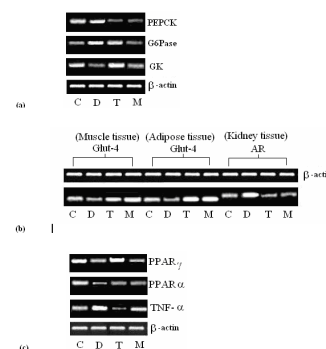


Fig. 5: RT-PCR analysis of (a) liver mRNA expression of glucokinase, glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (b) muscle and adipose mRNA expression of Glut-4 and kidney mRNA expression of aldose reductase (c) adipose tissue mRNA expression of PPAR γ , PPAR α and TNF- α genes, from control, diabetic, pterostilbene and metformin treated groups. GK, glucokinase; G6Pase, glucose-6-phosphatase; PEPCK, phosphoenolpyruvate carboxykinase; PPAR γ , peroxisome proliferators activated receptor γ ; PPAR α , peroxisome proliferators activated receptor α ; TNF- α , tumor necrosis factor- α ; Glut-4, glucose transporter-4; AR, aldose reductase. C, control; D, diabetic; T, pterostilbene treated; M, metformin treated.

hand, upon treatment with pterostilbene, there was no sign of steatosis (Fig. 7d to f).

In-vitro Analysis of Anti-diabetic Potential of Pterostilbene

Glucose Uptake by the Muscle Tissues

Stimulation of glucose uptake by psoas muscle in diabetic rats was studied by measuring the decrease in glucose concentration in the incubation medium with time. Incubation in the presence of pterostilbene (100 mg/L) resulted in about 47% increase in glucose uptake up to 30 minutes by itself, after which it continued further till 150 minutes as tested by us (Table 3). However, in the presence of insulin, increase in glucose uptake was not improved beyond the effect shown by the phytochemical alone (6%) which could be attributed to the fact that the tissues were insulin resistant.

Peroxisome Proliferators-activated Receptor Transactivation Assay

To test if pterostilbene could induce the transactivation of PPAR, a luciferase-based transactivation assay was designed using 3T3-L1 preadipocyte cells. As shown in Fig. 8, pterostilbene induced the activation of both PPAR α and PPAR γ in a dose-dependent manner albeit at significantly higher efficiency for PPAR α . Pterostilbene resulted in significant up-regulation for both PPAR α and PPAR γ from 10 and 20 mg/L concentrations, respectively, which continued further till 50 mg/L (42 and 20% transactivation of PPAR α and PPAR γ respectively), after which it leveled off (Fig. 8). This further conclusively proved that although pterostilbene has the capacity for dual transactivation of both PPAR α and PPAR γ it is more efficient as a ligand for the former than the latter.

Effects on Adipocytes Differentiation

Incubation of 3T3-L1 preadipocytes with pterostilbene resulted in a clear inhibition of adipocytes differentiation as evident from oil O red staining (Fig. 9a). Significant decrease in cellular triglyceride content was also observed in the treated cells as reported by other authors also^[16] compared to the positive control rosiglitazone as well as vehicle-treated control (Fig. 10b) ($p < 0.05$). This further confirmed the role of this phytochemical in treating obesity and insulin resistance.

DISCUSSION

Diabetes is a chronic metabolic disorder affecting a major population worldwide. A sudden switch from basic lifestyle to the so-called western lifestyle and other genetic and environmental factors result in obesity, hyperlipidemia, and hypertension. A diet containing high-fat constituents results in an elevated plasma-free fatty acid level which further causes a systemic insulin resistance. Insulin

resistance in peripheral tissues like skeletal muscles and adipose tissue ameliorates the ability to clear glucose from systemic blood flow.^[17] Accumulation of lipid in liver due to high plasma free fatty acid level inhibits glucose uptake and glycogen storage, at the same time this results in an enhanced gluconeogenesis and increased glucose output from the liver.^[18] All these factors collectively increase blood glucose level, i.e. hyperglycemia, a root cause of multiple complications associated with diabetes.

In the present study pterostilbene has shown its effects in four basic organs i.e., skeletal muscle, adipose tissue, liver, and pancreas organs, directly or indirectly. Skeletal muscle is quantitatively the most important tissue involved in systemic glucose clearance, and incubation of muscle tissue in the presence of pterostilbene isolated from *P. marsupium* further improved its efficiency of glucose uptake. Improvement in glucose disposal could be attributed to the enhanced expression profile of Glut-4 in muscle tissue compared to its diabetic counterpart. An

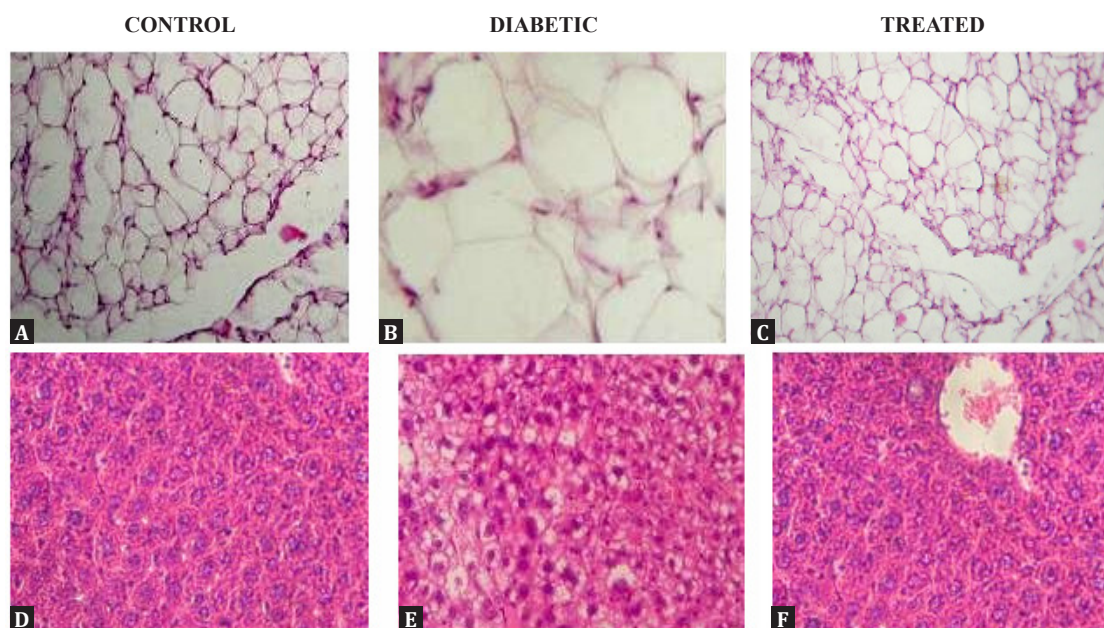


Fig. 7: Photomicrograph of the sections from the adipose (a-c), and liver tissues (d-f) of various groups of animal. The arrow indicates the islet region of the section. Haematoxylin and Eosin, 20X objective

Table 3: Effect of *pterostilbene* on glucose uptake from medium by psoas muscle isolated from high fat diet induced diabetic rats. The data are represented as increase in the cellular level of glucose with time, implying the equivalent reduction in glucose level in media by cellular uptake.

Treatment group	Glucose uptake (mg/dL)				
	30 minutes	60 minutes	90 minutes	120 minutes	150 minutes
MT	17.53 ± 0.43	25.80 ± 1.35	32.87 ± 0.82	39.20 ± 0.75	42.37 ± 0.96
MT + P	25.73a ± 0.43 (47)§	33.73 a ± 0.92 (31)§	38.40 a ± 0.98 (17)§	52.90 a ± 0.74 (35)§	65.30 a ± 0.84 (54)§
MT + I	20.90 ± 1.11	24.40 ± 0.51	31.70 ± 0.86	37.57 ± 0.49	43.73 ± 0.99
MT + I + P	27.23 ± 0.55 (6)γ	34.67 ± 0.78 (3)γ	41.77 ± 0.99 (9)γ	53.37 ± 0.90 (4)γ	67.83 ± 1.04 (4)γ

Values are mean ± S.E.M. of three independent experiments each performed in quadruplicates. MT: muscle tissue; P: *pterostilbene*; I: insulin. *Pterostilbene* and insulin in the incubation was added at a concentration of 100 and 25 mg/l, respectively.

§ values in the bracket indicate percent increase when compared with muscle tissue alone at that particular time point.

γ values in the bracket indicate percent increase when compared with muscle tissue with extract at that particular time point.

a, $p < 0.05$ when compared to control (only MT).



earlier report demonstrated that resveratrol (an analogue of pterostilbene) augmented glucose uptake in muscle cells by the overexpression of Glut-4.^[8]

Pterocarpus marsupium is a traditional medicinal plant known for its lipid lowering effects.^[10] Results from our study further confirmed it by demonstrating its efficacy in the lowering of TG and total cholesterol (TC) of type II diabetic animals. This was further confirmed by adipocytes differentiation assay where incubation of preadipocytes with pterostilbene resulted in a marked inhibition of differentiation.

Triglyceride accumulation in liver tissue results in increased gluconeogenesis, glycogenolysis, reduced glycogen synthesis and contributed further in hyperglycemic condition. Treatment with pterostilbene for eight weeks resulted in reduced hepatic lipid accumulation probably due to improved liver lipid metabolism and reduced plasma triglyceride content. High glucose-6-phosphatase and low hexokinase activities are characteristic features of insulin resistance, resulting in hyperglycemia.^[19] Treatment with pterostilbene improved glucoregulation, which could be attributed to the increased expression of glucokinase, a key regulator for glycolysis and increased glucose utilization as an energy source. Further, decreased activity of glucose-6-phosphatase, a key regulator in gluconeogenesis, reduces glucose production from the liver and counteracts the hyperglycemic condition of diabetes. In addition, a marked reduction in the expression of aldose reductase in response to pterostilbene confirmed that this phytochemical also plays in a major role in reducing diabetes-related complications.

Improvement in peripheral glucose tolerance is the key feature of pterostilbene treatment, which is evident from the sharp decline of blood glucose level during

OGTT, which could be attributed to the improved insulin sensitivity due to lower plasma lipid profile reduced free fatty acid-induced insulin resistance in the peripheral system. A clear improvement in Glut-4 also contributes to improved glucose uptake from peripheral tissues. Similar results were observed from grape seed-derived resveratrol in soleus muscle of streptozotocin-induced diabetic rats, where treatment resulted in enhanced Glut-4 expression contributing to the anti-hyperglycemic effects of resveratrol.^[20] Increased expression of Glut-4 and at the same time the reduced expression of TNF- α explains the improvement in insulin-resistant condition.

The activity of PPAR α and PPAR γ showed significant improvement during different levels of analysis. Due to their insulin receptor sensitizing activity, the agonist for these receptors (thiazolidinediones) is used to treat type II diabetes mellitus. In our study, the transactivation assays showed clearly that pterostilbene is a potent activator for both PPAR α and PPAR γ albeit to a lesser extent for the latter. Dual regulations of PPAR α and PPAR γ have been reported earlier by several other plant extracts as well.^[21,22] PPAR α is predominantly expressed in tissues catabolizing high amounts of fatty acids, such as liver, heart, and brown adipose tissue and controls the expression of various genes involved in intra- and extracellular lipid metabolism acyl-coA oxidase, acyl-coA synthetase, and apolipoproteins A-I, A-II and C-III. Since PPAR γ specific agonists are associated with extensive weight gain, co-activation of PPAR α using a separate agonist or dual activator has been proposed to be an effective remedy for this side effect.^[23,24] This effect of pterostilbene could be attributed to the dual nature of this phytochemical as PPAR α and PPAR γ agonist, where the former controls the overall lipid metabolism

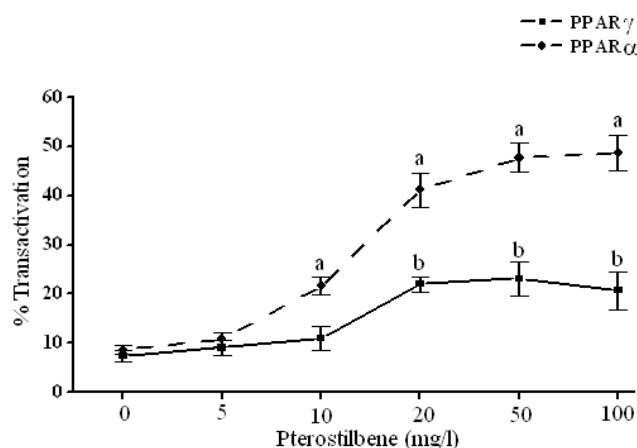


Fig. 8: Transactivation assay to determine the PPAR activation. Values are means $\pm$ S.E.M. of five independent experiments each performed in quadruplicates and are expressed as percentage of the positive controls for PPAR α (Wy 14643, 16.2 mg/L) and PPAR γ (Rosiglitazone, 1.8 mg/L) which were given a value of 100% respectively. a and b, indicates the significant level of difference in values as compared to vehicle treated control incubation for PPAR α and PPAR γ respectively ($p < 0.05$).

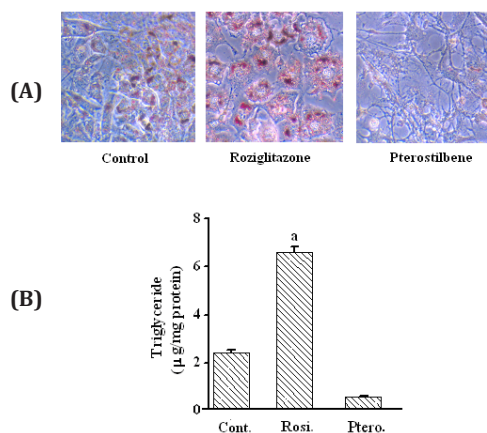


Fig. 9: Effect of pterostilbene on 3T3-L1 preadipocyte differentiation. (a) The cells stained with oil red O at day 10 after treating with 50 mg/L of pterostilbene and visualized at 20X magnification. (b) The cellular TG content expressed as μ g/mg of protein. Data are expressed as mean $\pm$ S.E.M. of three independent experiments each performed in quadruplicates. a, represents the significant level of effect as compared to vehicle treated control groups ($p < 0.05$). Cont., vehicle treated control; Rosi., rosiglitazone treated; Ptero., pterostilbene treated.

and counteracts the weight gaining nature of the latter agonists.

We further investigated the effect of pterostilbene on serum insulin level. It was found that pterostilbene caused a marked reduction in serum insulin level, which agreed with earlier reports where resveratrol showed similar effects. A temporary β -cell rest has been associated with at least partial amelioration of their endocrine function in the future. The antihyperglycaemic activity of pterostilbene is thus due to the release of insulin from the existing β -cells of the pancreas.^[25]

CONCLUSION

It is evident from the above results and observations that pterostilbene can act on various critical nodes of carbohydrate and lipid metabolism pathways. To the best of our knowledge, this is the first-ever report on this phytochemical, which has been purified from a plant source and analyzed to obtain a comprehensive idea about its mode of action unlike reported by others using pure synthetic chemical. The present data thus provides a basis for further detailed analysis on this phytochemical's structure-function cross talk to elucidate its exact mode of action before it could be used extensively for the management of diabetes.

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