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Research on effect of Pterostilbene on hepatic key enzymes of glucose metabolism.

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ABSTRACT
The purpose of this study was to investigate the effect of pterostilbene and its effect on key enzymes of glucose metabolism. Diabetic rats were orally administered with pterostilbene (10,20.40,500mg/kg) for 42 days fro 22nd onword everyday for 42 day viz day 63on glucose was determined. Administration of pterostilbene at 40 mg/kg significantly decreases plasma glucose. Based on these data, the higher dose, 40 mg/kg pterostilbene, was selected for further evaluation. Administration of pterostilbene for 42 dys on glucose, insulin levels and hepatic enzymes in normal and streptozotocin (STZ)-high fructose diet(HFD)-induced diabetic rats. A significant decrease in glucose and significant increase in plasma insulin levels were observed in normal and diabetic rats treated with pterostilbene. Treatment with pterostilbene resulted in a significant reduction of glycosylated hemoglobin and an increase in total hemoglobin level. The activities of the hepatic enzymes such as hexokinase was significantly increased whereas glucose-6-phosphatase, fructose-1,6-bisphosphatase were significantly decreased by the administration of pterostilbene in diabetic rats. A comparison was made between the action of pterostilbene and the antidiabetic drug metformin.
Keywords: Pterostilbene, Antidiabetic, hepatic key enzymes, Experimental diabetes.

I. INTRODUCTION

The word diabetes comes from Latin diabetes, which in turn comes from Ancient Greek (diabenin) which literally means "a passer through; a siphon". Indian physicians identified the disease and classified it as **madhumeha** or "**honey urine**", noting the urine would attract ants. The disorder is also known as '**Prameha**', which means watering. In relation to human disease it may have a meaning of passing urine, qualified by prefix 'pra' meaning excess in both frequency and quantity and 'meha' meaning urination (Douglas et al., 2011; John et al., 2011). **Diabetes** is any disorder of metabolism causing excessive thirst and production of large volume of urine (Harrison, 1986). The number of obese individuals increases worldwide, leading to consider obesity as a global epidemic. It has been estimated that, in 2005, approximately 1.6 billon adults were overweight and more than 400 million adults were obese. Nowadays, around 15 % of the world's population is overweight or obese, according to the International Association for the Study of Obesity (website: http://www.iaso.org). Obesity is recognized as a major risk factor for type 2 diabetes, cardiovascular diseases, non-alcoholic fatty liver diseases and certain types of cancers. Insulin resistance (namely the deficiency of response to insulin, leading to much blunted glucose uptake into cardiac or skeletal muscles and adipose tissues) is a metabolic disturbance that firmly links excess of white adipose tissue (WAT) with the obesity-related complications.

Diabetes Inspidus: It is a rare metabolism disorder in which the patient produces large quantities of dilute urine and due to deficiency of ADH (anti diuretic hormone) or vasopressin hormone (Harrison, 1986). **Diabetes mellitus (DM):** Diabetes mellitus defined as a heterogeneous metabolic disorder characterized by chronic hyperglycemia with disturbance of carbohydrates, fat and protein metabolism. Sustained hyperglycemia has been shown to affect almost all tissues in the body and is associated with significant complications of multiple organ systems, including the eyes, nerves, kidneys and blood vessels. It has emerged as the major cause of adultmorbidity and mortality worldwide (Gulliford, 1994).

Diabetes mellitus is a serious condition with potentially devastating complications that affects all age groups worldwide. In 1985, an estimated 30 million people around the world were diagnosed with diabetes; in 2000, that figure rose to over 150 million; and, in 2012, the International Diabetes Federation (IDF) estimated that 371 million people had diabetes. That number is

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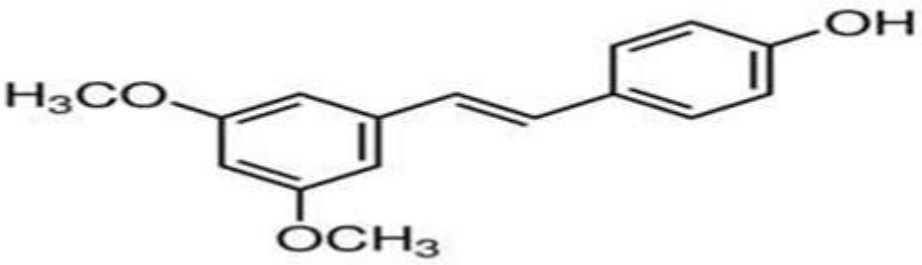
projected to rise to 552 million by 2030.



(Structure of Pterostilbene)

Pterostilbene (trans-3,5-dimethoxy-4-hydroxystilbene) is a naturally derived compound found primarily in blueberries and Pterocarpus marsupium (PM) heartwood (Roupe KA, et.al,2006). The amount of daily pterostilbene consumption varies according to dietary fruit intake, and it has been estimated that pterostilbene content per blueberry varies from 99 ng to 520 ng/gram depending on the type of berry ingested (Rimando AM et.al, 2004). Substantial evidence suggests that pterostilbene may have numerous preventive and therapeutic properties in a vast range of human diseases that include neurological, cardiovascular, metabolic, and hematologic disorders(McCormack D, et.al) Pterostilbene is structurally similar to resveratrol, a compound found in red wine that has comparable antioxidant, anti- inflammatory,anti-diabetic and anticarcinogenic properties; however, pterostilbene exhibits increased bioavailability due to the presence of two methoxy groups which cause it to exhibit increased lipophilic and oral absorption (Stivala LA, et.al 2001) In animal studies, pterostilbene was shown to have 80% bioavailability compared to 20% for resveratrol making it potentially advantageous as a therapeutic agent (Kapetanovic et.al 2011).

Pterostilbene in diabetes:

The heartwood of the plant Pterocarpus marsupium (Pterostilbene) has been shown to exhibit antiglycemic properties in multiple studies. In a study performed by (Grover et al,2005) rats were fed high-fructose diets to induce hyperglycemia and insulin resistance and then treated with PM orally for thirty days (Grover JK et al,2005). The authors hypothesized that Pterostilbene treatment would counteract the metabolic side effects of a high-fructose diet by mitigating hyperglycemia, hyperinsulinemia and hypertriglycemia. Results of the study show that rats fed high-fructose diets combined with Pterostilbene treatment had lower levels of hyperinsulinemia, hypertriglycemia, and complete prevention of hyperglycemia. It has been hypothesized that the antiglycemic properties possessed by PM are attributed to pterostilbene. Experiments performed by (Manickam et al,1997) and colleagues assessed the antiglycemic effects of pterostilbene isolated from Pterostilbene in a STZ induced rat model of hyperglycemia and found that oral dosing of pterostilbene significantly decreased plasma glucose levels by 42%. The proposed mechanism for the antidiabetic effects exerted by pterostilbene is reduction of OS, which plays a critical role in aberrant glucose regulation. (Satheesh et al.2006) hypothesized that pterostilbene treatment in diabetic rats would increase antioxidant activity and lessen the impact of OS on kidney and liver cells. The experimental design measured OS using thiobarbituric acid reactive substance (TBARS) and hydroperoxide (HP) levels in diabetic rats treated with 40 mg/kg oral pterostilbene in comparison to diabetic rats treated with Metformin. The authors also evaluated the effect of pterostilbene upon the activity of antioxidant enzymes catalase, SOD, GPx, and GST. Results of the experiments show that DM control rats exhibited marked increases in TBARS and HP in liver and kidney tissue that was subsequently inhibited by pterostilbene treatment (Satheesh et al.2006). In diabetic rats, pterostilbene decreased TBARS by 61.5% and 33.3% in liver and kidney tissue, respectively.

II. MATERIALS AND METHODS:

Animals:

A total of 48 Sprague-Dawley rats (weight

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range: 200-250 g) were procured from Piramal Healthcare Ltd (Goregaon, Mumbai) and placed in the experimental room (SADAR PATEL UNIVERSITY OF BALAGHAT). The experimental room was maintained under standard conditions of temperature (25°C ± 2°C) and relative humidity (55 ± 10%). Animals were subjected to 12h light and dark cycle. Animals were housed in standard polypropylene cages with wire mesh top and husk as bedding and allowed to acclimatize for one week prior to commencement of experiment. During this period animals were fed with commercially available rodent food pellets and water ad libitum. All the experimental procedures were approved with the protocol (CPCSEA/BCP/2014- by the Institutional Animal Ethics Committee (IAEC)) of Bombay College of Pharmacy and were carried out in accordance with the current guidelines for the care of laboratory animals.

Chemicals:

Streptozotocin (STZ) was purchased from SRL chem.Ltd.,Mumbai,India. Pterostilbene was received fro m Nanjing Zelang medical technology co. Ltd, china. All the other chemicals and reagents used were of analytical grade.

Induction of Diabetes:

Animals weighing in the range of 200-250 g were selected and had access to 10% fructose solution in drinking water bottles. The rats were rendered diabetic by a single intraperitoneal dose of 40 mg/kg of STZ freshly dissolved in ice cold 0.1 M citrate buffer (pH 4.5) on day 15 (Hemalatha et al. 2004). After 1 week , fasting blood glucose (FBG) levels were measured and only those animals showing blood glucose level more than 250 mg/dl were randomly allocated to groups (Ewart et al., 1975).

Treatment schedule and Estimation of fasting blood glucose (FBG) level:

After induction of experimental diabetes, the rats were divided into six groups (n=8). Except for group I, which served as normal (non-diabetic) control, all other groups were comprised of diabetic rats. Group II served as diabetic (STZ) Positive control. Groups III, IV and V received.

Pterostilbene (10, 20 and 40 mg/kg respectively) and group VI received reference/standard drug Metformin (500 mg/kg) daily for 42 days from day 22nd onwards, everyday. For 42 day viz day 63.

Grouping Of Rats	
Group I (Normal control)	Normal Rats (Normal control)
Group II (Diabetic control)	Diabetic Rats (Positive control)
Group III (Test low dose)	Diabetic Rats + Pterostilbene (10 mg/kg), p.o. in 1% CMC
Group IV (Test medium dose)	Diabetic Rats + Pterostilbene (20 mg/kg), p.o. in 1% CMC
Group V (Test high dose)	Diabetic Rats + Pterostilbene (40 mg/kg), p.o. in 1% CMC
Group VI (Standard)	Diabetic Rats + Metformin (500 mg/kg), p.o. in 1% CMC

Treatment regimens:

48 Rats were provided with fructose 10% solution in drinking water ad libitum for 14 days. Streptozotocin in citrate buffer pH 4.5 was injected intraperitoneally on day 15th. One week after the Streptozotocin injection, on the 21st day blood glucose level were measured. The rats with non fasting blood glucose more than 250 mg/dl were considered as diabetic and allocated randomly to all treatment groups. They were divided into 6 groups as follow:

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Group No.	Treatments	Dose (mg/kg)/ percentage	Route of administration	No. of rats	Treatment(days)
1.	Vehicle control citrate buffer (1%CMC)	--	i.p. oral	8	22 to 63 days
2.	Fructose solution Streptozotocin	10% 40	In drinking water i.p. (in citrate buffer)	8	1 to 14 days 15th day
3.	Fructose solution Streptozotocin Pterostilbene	10 % 40 10	In drinking water i.p. (in citrate buffer) p.o. in 1% CMC	8	1 to 14 days 15th day 22 to 63 days
4.	Fructose solution Streptozotocin Pterostilbene	10 % 40 20	In drinking water i.p. (in citrate buffer) p.o. in 1% CMC	8	1 to 14 days 15th day 22 to 63days
5.	Fructose solution Streptozotocin Pterostilbene	10 % 40 40	In drinking water i.p. (in citrate buffer) p.o. in 1% CMC	8	1 to 14 days 15th day 22 to 63days
6.	Fructose solution Streptozotocin Metformin	10 % 40 500	In drinking water i.p. (in citrate buffer) p.o. in 1% CMC	8	1 to 14 days 15th day 22 to 63days

The respective treatments were initiated from the 22nd day, each day till the 63rd day. On the 63rd day of treatment, overnight fasted rats shall be mildly anesthetized with ketamine (60 mg/kg) and xylazine (6 mg/kg) (10:1) combination intraperitoneally. 0.5 ml blood will be collected by retro-orbital route using alternative eyes at each of 0, 30, 60, 90,120 and 180 min time intervals for oral glucose tolerance test and estimating serum insulin.

The animals were then euthanized using solid carbon dioxide chamber, blood will be collected from abdominal aorta to estimate various biochemical markers. The in vitro,

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histopathological studies shall be carried out in isolated pancreas and liver.

Evaluation:

Food and water intake was recorded daily whereas weight of animals was recorded thrice weekly, meanwhile anthropometrical analysis was performed on 1st day (initial), on 32nd day (intermittent) and 63rd day (terminal) to determine the effects of treatment interventions. On 63rd day from the start of treatment, animals were fasted 8-12 hours at night and blood was withdrawn by retro-orbital plexus at various time points for oral glucose tolerance test and fasting insulin.

Euthanasia:

After completion of 9 weeks of treatment period, animals were sacrificed with solid CO_2 and blood was withdrawn from abdominal aorta for determination of other biochemical parameters. Animals were dissected. Liver was perfused and stored at -20°C. The Liver and Pancreas were examined. Blood samples were kept in ice-bath for 1 hr and then centrifuged at 5000 rpm for 15 min at 4°C. Serum aliquots were stored at -20°C until assayed for serum glucose, triglyceride, total cholesterol, and insulin level and for other Biochemical parameters.

BIOCHEMICAL ANALYSIS:

1) Oral Glucose Tolerance Test:

Rats were fasted 8-12 hours at night. An oral glucose tolerance test (OGTT) was performed usingglucose of 2 g/kg of body weight of rats, administered using oral gavage.Blood samples were collected by retro-orbital routeand taken at 0 min, 30, 60, 90, 120 and 180 min of glucose administration.

Calculations:

Table shows that serum glucose was increased significantly in the positive control group when compared with normal control ($p<0.05$). Also at 3 hr after glucose load the glucose concentration in the positive control group was significantly higher in the positive control group when compared with normal control group. The AUC glucose (0-180 min) was greatest in the Positive control group (47217.18 ± 5493.13) as compared to Normal control group (25798.7 ± 6478.5). The AUC glucose (0-180 min) of 10 mg/kg (40638.18 ±3485.73), 20 mg/kg (43362.79 ±3871.85) or 40 mg/kg (45202.41 ± 41202.46) and 500 mg/kg Metformin (39473.42 ± 4981.5) was significantly lower as compared to Positive control group.

Impact of Pterostilbene on OGTT in Streptozotocin and HFD Induced diabetic rats.

Blood glucose (mg/dl)						
Groups	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**	**180 min**
Normal control	121.8±6.7	251.14±14	211.85±59	225.8±57.5	200.28±68.5	144.4±8.8
Positive control	111.8±4.3*	309.3±33*	365.3±26.9*	402.0±21.3*	335.1±30.5*	212.6±24.5*
Pterostilbene (10 mg/kg)	111.5±3.3#	289.0±29.8#	329.0±24.6#	353.1±28.3#	255.8±16.8#	140.1±9.96#
Pterostilbene (20 mg/kg)	110.1±4.8#	306.8±32.1#	346.1±29.8#	373.8±23.8#	276.8±24.2#	172.3±16.9#
Pterostilbene (40 mg/kg)	100.8±5.2#	273.2±42.0#	287.8±47.7#	251.8±43.5#	224.2±34.8#	172.0±24.5#
Metformin (500 mg/kg)	109.3±3.7#	295.6±19.0#	300.33±28#	315.5±12.9#	266.3±26.9#	166.8±14.4#

Values are expressed as mean ± SEM (n=8), statistical analysis performed by 2- way ANOVA with time and treatment as variable. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

Serum glucose levels were significantly higher in Positive control group as compared to Normal control group. Pterostilbene was found to prevent the elevations in blood glucose levels in 10, 20, or 40 mg/kg all the dose of Pterostilbene. This response was cofounded on Metformin treatment. Blood glucose levels of all the doses of Pterostilbene & Metformin shows stastically significance with the Positive control group.

2) Serum glucose (mg/dl):

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Impact of Pterostilbene on Blood Glucose Levels in Streptozotocin and HFD Induced diabetic rats.

Groups	**63rd day Serum glucose (mg/dl)**
Normal control	102.16 ± 2.086
Positive control	410.50 ± 11.88*
Pterostilbene (10 mg/kg)	192.80 ± 5.784#
Pterostilbene (20 mg/kg)	181.19 ± 5.984#
Pterostilbene (40 mg/kg)	143.12 ± 5.342#
Metformin (500 mg/kg)	**165.76 ± 4.310#**

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control. #p <0.05 when compared to Positive control group.

(3) Lipid Profile Parameters:

(3.1)Serum triglycerides (mg/dl):

A significant rise in Serum triglycerides level was observed in positive control group as compared to normal control group. Pterostilbene was found to avert the elevated levels of triglycerides in 10, 20, 40 mg/kg dose of Pterostilbene dose, this was observed in Metformin 500mg/kg treatment group. Serum triglycerides levels of all the doses of Pterostilbene and Metformin show stastically significant drop as compared with the Positive control group.

Groups	Serum triglyceride (mg/dl)
Normal control	88.00±2.37
Positive control	300.57±7.34*
Pterostilbene (10 mg/kg)	242.01±4.81#
Pterostilbene (20 mg/kg)	183.86±10.88#
Pterostilbene (40 mg/kg)	111.39±7.98#
Metformin (500 mg/kg)	117.71±13.60#

Impact of Pterostilbene on Serum triglyceride Levels In Streptozotocin and HFD Induced diabetic rats.

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control. #p <0.05 when compared to Positive control group.

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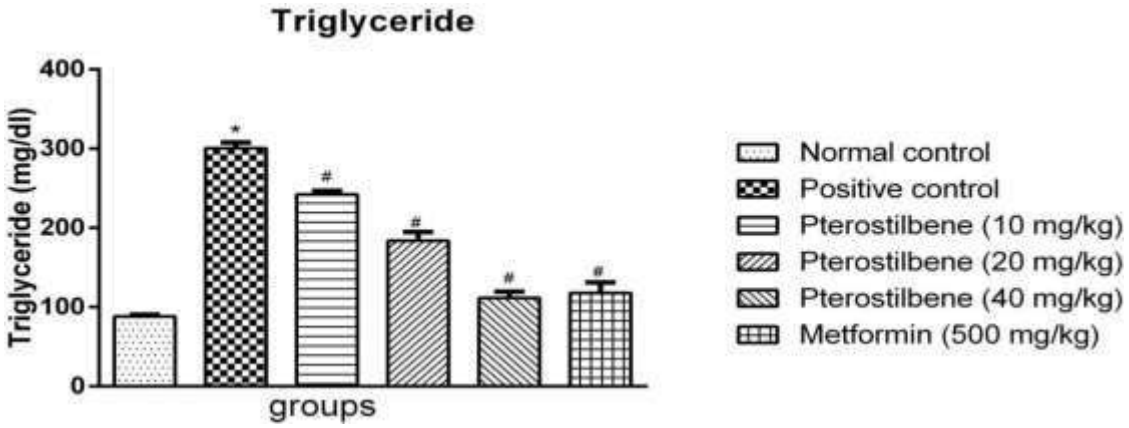

Impact of Pterostilbene on Serum triglyceride Levels In Streptozotocin and HFD Induced diabetic rats.

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control. #p <0.05 when compared to Positive control group.

A significant rise in serum cholesterol levels was observed in Positive control group as compared to Normal control group. Pterostilbene was found to avert the elevated serum cholesterol levels in 10, 20, 40 mg/kg of Pterostilbene dose as well as in Metformin 500mg/kg. Serum cholesterol levels of animal treated with all the doses of Pterostilbene and Metformin shows stastically significant decrease with the Positive control group.

(3.2) Serum cholesterol (mg/dl):

Impact of Pterostilbene on Total Cholesterol Levels in Streptozotocin and HFD Induced diabetic rats.

Groups	Total cholesterol (mg/dl)
Normal control	91.70±5.15
Positive control	197.12±9.14*
Pterostilbene (10 mg/kg)	180.10±15.42#
Pterostilbene (20 mg/kg)	166.89±20.48#
Pterostilbene (40 mg/kg)	102.37±11.56#
Metformin (500 mg/kg)	105.16±10.45#

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

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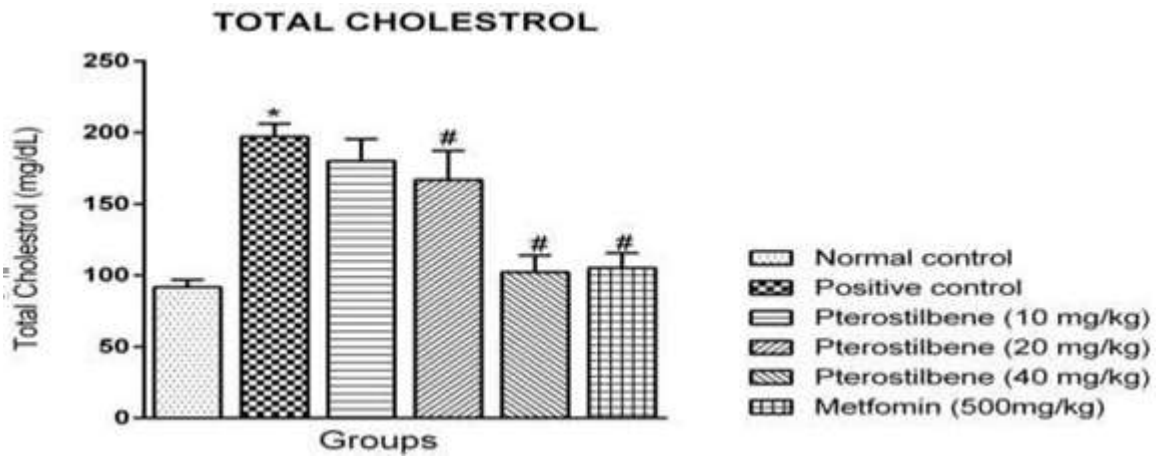

Impact of Pterostilbene on Total Cholesterol Levels in Streptozotocin and HFD Induced diabetic rats.

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

(3.3) Serum HDL (mg/dl):

Positive control group exhibited significantly lower HDL as compared to Normal control group. Treatment with Pterostilbene at a dose of 10, 20 or 40 mg/kg and 500 mg/kg Metformin significantly elevate the reduce HDL level**.**

Impact of Pterostilbene on HDL levels in Streptozotocin and HFD Induced diabetic rats.

Groups	HDL (mg/dl)
Normal control	64.95±0.966
Positive control	38.56±0.665*
Pterostilbene (10 mg/kg)	40.19±1.082
Pterostilbene (20 mg/kg)	49.15±0.845#
Pterostilbene (40 mg/kg)	54.02±1.061#
Metformin (500 mg/kg)	51.42±0.704#

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

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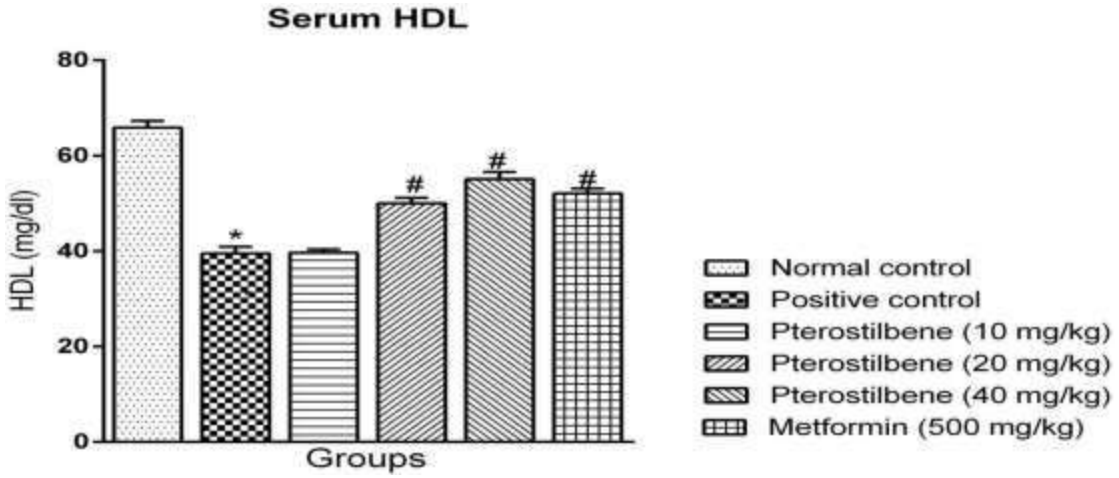

Positive control group exhibited significantly lower HDL as compared to Normal control group. Treatment with Pterostilbene at a dose of 10, 20 or 40 mg/kg and 500 mg/kg Metformin significantly elevate the reduce HDL level.

(3.4) Serum VLDL (mg/dl):

A significant rise in VLDL levels was observed in Positive control group as compared to Normal control group. Pterostilbene was found to avert the elevated VLDL levels in 10, 20 or 40 mg/kg of Pterostilbene dose as well as in Metformin 500mg/kg. VLDL levels of all the doses of Pterostilbene & Metformin shows stastically significance with the Positive control group.

Impact of Pterostilbene on VLDL in Streptozotocin and HFD Induced diabetic rats.

Groups	VLDL
Normal control	17.60±2.48
Positive control	60.11±4.82*
Pterostilbene (10 mg/kg)	48.40±5.19$^{\#}$
Pterostilbene (20 mg/kg)	36.77±6.15$^{\#}$
Pterostilbene (40 mg/kg)	22.27±3.47$^{\#}$
Metformin (500 mg/kg)	23.65±2.87$^{\#}$

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. $^{\#}$p <0.05 when compared to Positive control group.

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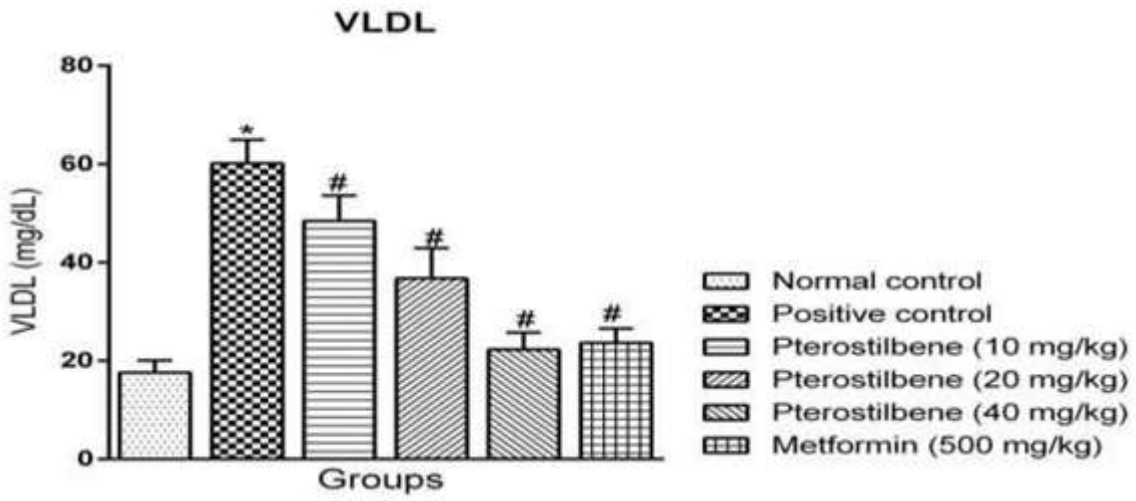

Impact of Pterostilbene on VLDL in Streptozotocin and HFD Induced diabetic rats.

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control. #p <0.05 when compared to Positive control group.

(3.5) Serum CREATININE (mg/dl):

Positive control group exhibited significantly higher Creatinine level as compared to Normal control group. Treatment with 10, 20 or 40 mg/kg Pterostilbene and 500 mg/kg Metformin significantly reduce the elevated Creatinine level

Impact of Pterostilbene on Creatinine in Streptozotocin and HFD diabetic rats.

Groups	CREATININE (mg/dl)
Normal control	0.5180±0.0039
Positive control	0.9032±0.0130*
Pterostilbene (10 mg/kg)	0.8020±0.0029#
Pterostilbene (20 mg/kg)	0.7824±0.0346#
Pterostilbene (40 mg/kg)	0.6373±0.0031#
Metformin (500 mg/kg)	0.5733±0.0219#

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followedby Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

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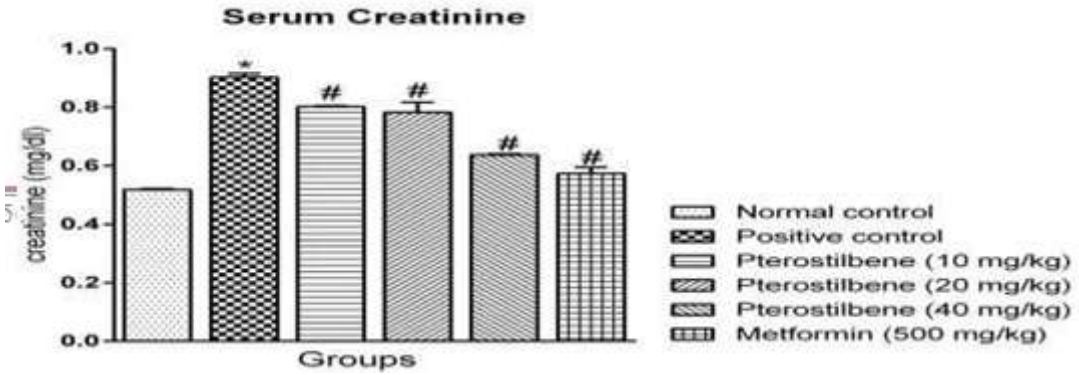

Impact of Pterostilbene on Creatinine in Streptozotocin and HFD Induced diabetic rats.

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

(4) Anthropometric parameters:

(4.1) Body weight (grams):

The body weight of normal and diabetic rats were summarized in table. The final body weights were significantly decreased in the diabetic control group as compared to normal control group. Administration of Pterostilbene atthe doses of 10, 20 or 40 mg/kg and 500 mg/kg Metformin significantly improve the body weight when compared to the diabetic control group.

Impact of Pterostilbene on body weight In Streptozotocin and HFD Induced diabetic rats.

Body weight in grams				
Groups	Week 0	Week 3rd	Week 6th	Week 9th
Normal control	220.8±9.21	241.66±7.31	263.83±12.84	289.5±11.8
Positive control	216.1±8.44	222.83±8.54	217.83±12.44	217.0±23.89
Pterostilbene (10 mg/kg)	219.5±6.15	234.83±8.44	222.16±9.92	218.5±21.2
Pterostilbene(20 mg/kg)	214.83±7.11	231.0±4.21	232.83±4.44	251.6±8.35
Pterostilbene(40 mg/kg)	223.8±5.41	241.5±4.32	240.6±3.38	266.1±8.0
Metformin(500 mg/kg)	216.0±6.22	230.16±4.8	234.16±5.60	254.50±6.86

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

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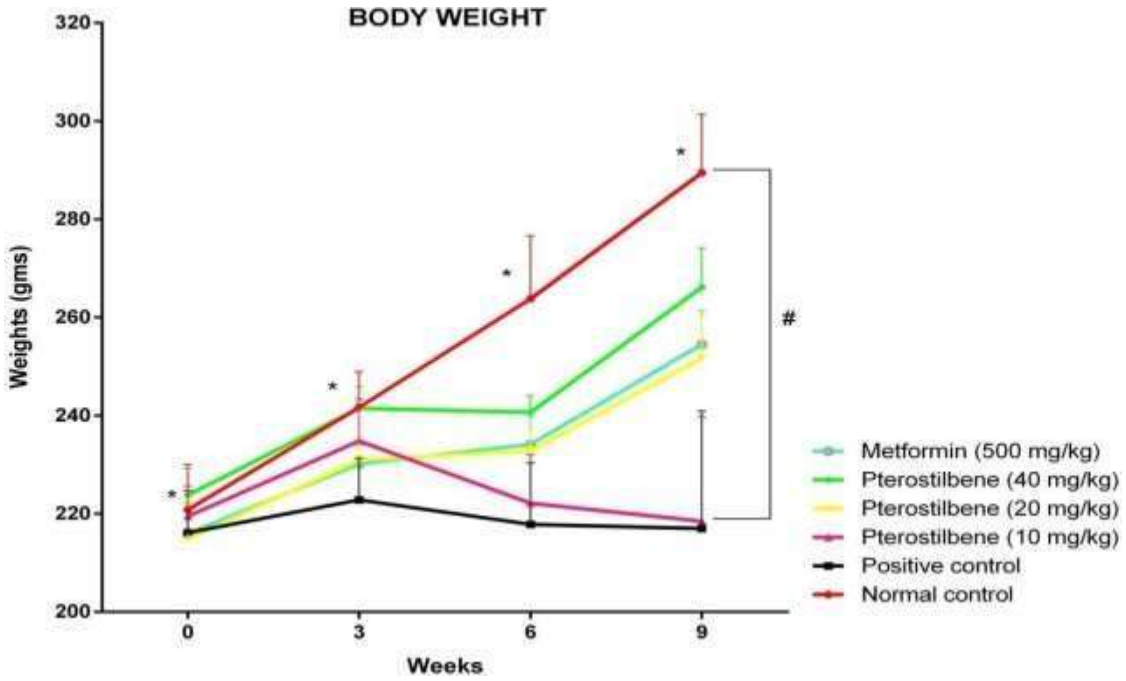

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. $^{\#}$p <0.05 when compared to Positive control group.

Body mass index in Positive control group was non-significantly lower as compared to normal control group. Treatment with Pterostilbene 10, 20 or 40 mg/kg and 500 mg/kg Metformin does not shows any significant difference when compared to the diabetic control group.

(4.2) BODY MASS INDEX (kg/m^2):

Impact of Pterostilbene on BMI in Streptozotocin and HFD Induced diabetic rats.

Groups	**BMI (kg/m^2)**
Normal control	0.521±0.008
Positive control	0.498±0.003*
Pterostilbene (10 mg/kg)	0.520±0.012
Pterostilbene (20 mg/kg)	0.480±0.006
Pterostilbene (40 mg/kg)	0.499±0.007
Metformin (500 mg/kg)	0.483±0.010

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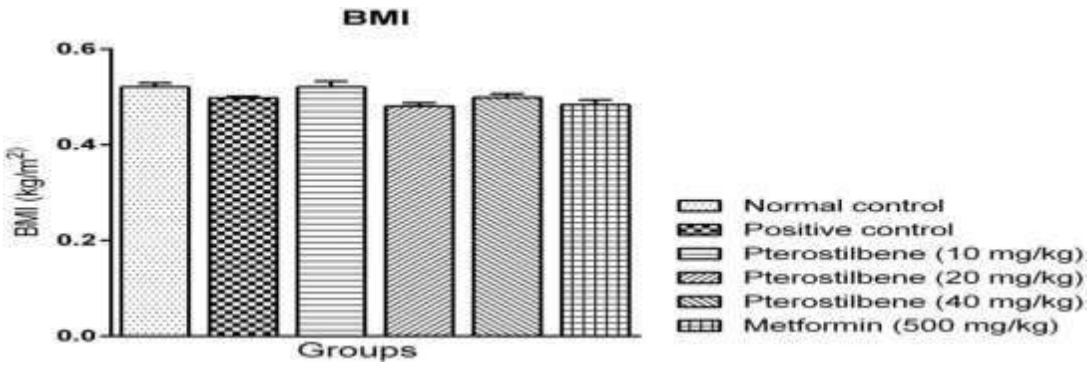
Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.



Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

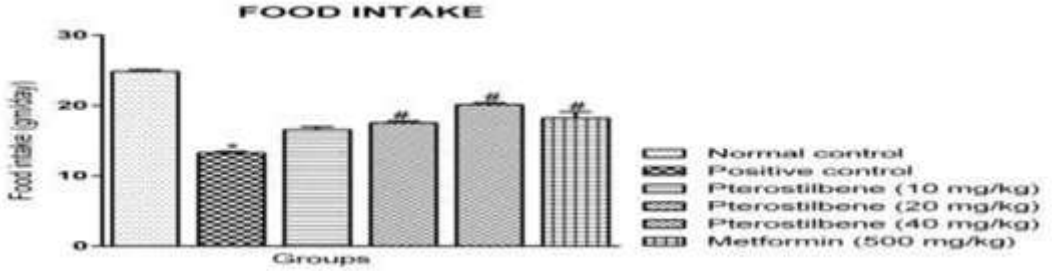
(4.3) FOOD INTAKE (gm/day):

Positive control group exhibited significantly lower consumption of food as compared to Normal control group. Treatment with 10, 20 or 40 mg/kg Pterostilbene and 500 mg/kg Metformin significantly improvethe consumption of food.

Impact of Pterostilbene on food intake in Streptozotocin and HFD Induced diabetic rats.

Groups	**Food intake (gm/day)**
Normal control	24.85±0.74
Positive control	13.25±0.65*
Pterostilbene (10 mg/kg)	16.56±1.20#
Pterostilbene (20 mg/kg)	17.56±0.67#
Pterostilbene (40 mg/kg)	20.14±0.83#
Metformin (500 mg/kg)	18.25±0.86#

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.



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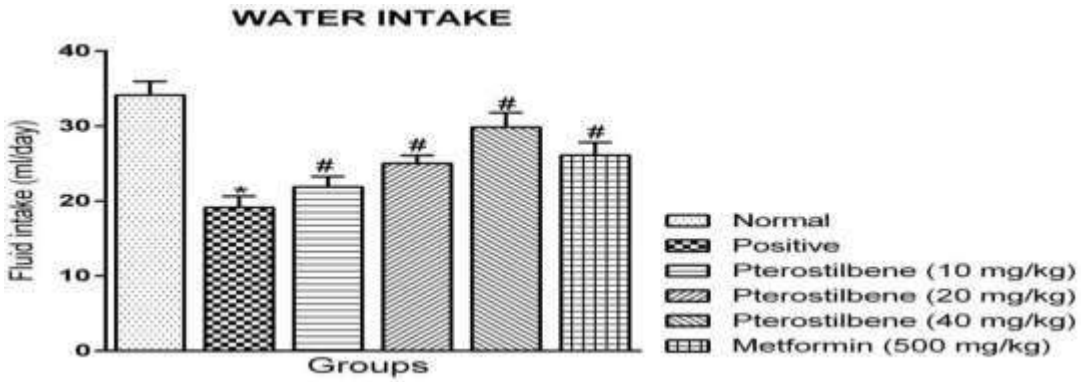
Impact of Pterostilbene on food intake in Streptozotocin and HFD Induced diabetic rats.
Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control. #p <0.05 when compared to Positive control group.

(4.4) WATER INTAKE (ml/day):
Positive control group exhibited significantly lower consumption of water as compared to Normal control group. Treatment with 10, 20 or 40 mg/kg Pterostilbene and 500 mg/kg Metformin significantly improvethe consumption of water.

Impact of Pterostilbene on water intake in Streptozotocin and HFD Induced diabetic rats.

Groups	Water intake (ml/day)
Normal control	34.12±1.82
Positive control	19.14±1.50*
Pterostilbene (10 mg/kg)	21.89±1.37#
Pterostilbene (20 mg/kg)	25.01±1.054#
Pterostilbene (40 mg/kg)	29.85±1.930#
Metformin (500 mg/kg)	26.10±1.722#

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.



Impact of Pterostilbene on water intake in Streptozotocin and HFD Induced diabetic rats.
Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

(4.5) LEE'S INDEX:
Lee's index in Positive control group was not-significantly lower as compared to normal control group. Treatment with Pterostilbene 10, 20 or 40 mg/kg and 500 mg/kg Metformin does not shows any significant difference when compared to the diabetic control group.

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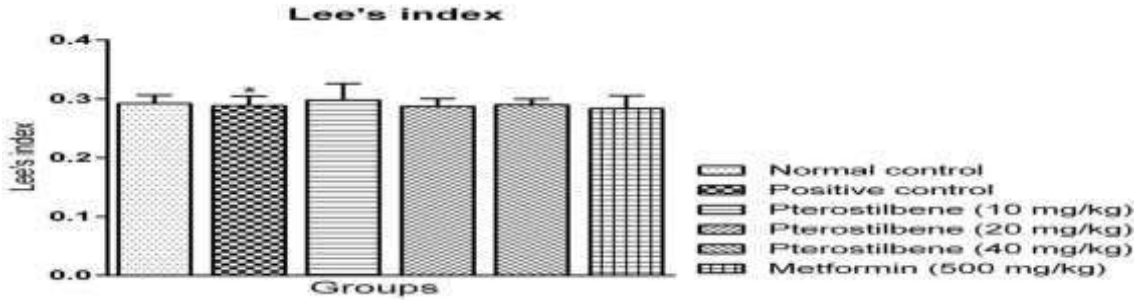
Impact of Pterostilbene on Lee's Index in Streptozotocin and HFD Induced diabetic rats.

Groups	LEE'S INDEX
Normal control	0.292±0.014
Positive control	0.288±0.016
Pterostilbene (10 mg/kg)	0.298±0.028
Pterostilbene (20 mg/kg)	0.287±0.013
Pterostilbene (40 mg/kg)	0.289±0.010
Metformin (500 mg/kg)	0.283±0.022

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.



Impact of Pterostilbene on Lee's Index in Streptozotocin and HFD Induced diabetic rats.
Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

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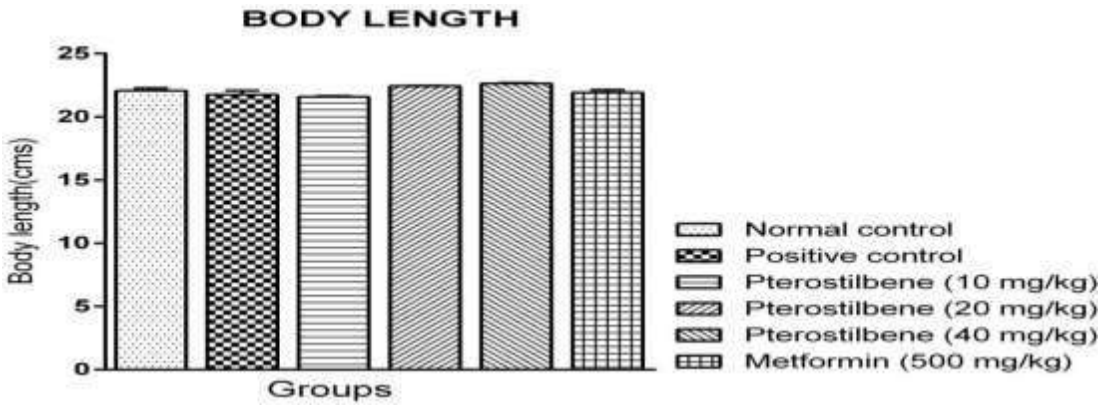
(4.6) Body length (cm):

There was no significant change in body length on induction with STZ and HFD. Treatment with Pterostilbene 10, 20 or 40 mg/kg and 500 mg/kg Metformin does not shows any significant.

Impact of Pterostilbene on body length in Streptozotocin and HFD Induced diabetic rats.

Groups	Body length (cm)
Normal control	22.08±0.067
Positive control	21.76±0.116
Pterostilbene (10 mg/kg)	21.59±0.023
Pterostilbene (20 mg/kg)	22.43±0.013
Pterostilbene (40 mg/kg)	22.63±0.024
Metformin (500 mg/kg)	21.92±0.075

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.



Impact of Pterostilbene on body length in Streptozotocin and HFD Induced diabetic rats.

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

(4.7) AC/TC RATIO:

There was no significant change in AC/TC ratio on induction with STZ and HFD. Treatment with Pterostilbene 10, 20 or 40 mg/kg and 500 mg/kg Metformin does not shows any significant.

Impact of Pterostilbene on AC/TC ratio in Streptozotocin and HFD Induced diabetic rats.

Groups	AC/TC RATIO
Normal control	1.150±0.011

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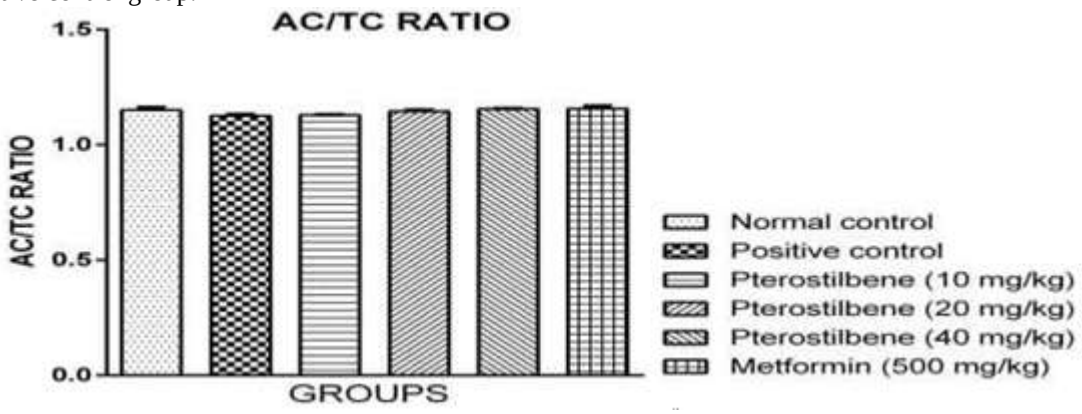
Positive control	1.126±0.006*
Pterostilbene (10 mg/kg)	1.129±0.002
Pterostilbene (20 mg/kg)	1.145±0.006
Pterostilbene (40 mg/kg)	1.155±0.002
Metformin (500 mg/kg)	1.156±0.011

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.



Impact of Pterostilbene on AC/TC ratio in Streptozotocin and HFD Induced diabetic rats.
Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

(5) Glycosylated hemoglobin (%):

Glycosylated hemoglobin level in Positive control group was significantly higher as compared to normal control group. Treatment with Pterostilbene (10, 20 or 40 mg/kg) and Metformin 500 mg/kg significantly reduced the HbA1c level when compared to the diabetic control group.

Impact of Pterostilbene on Glycosylated hemoglobin in Streptozotocin and HFD Induced diabetic rats.

Groups	**Glycosylated hemoglobin (%)**
Normal control	5.426±0.019
Positive control	14.70±0.811*
Pterostilbene (10 mg/kg)	16.61±0.059
Pterostilbene (20 mg/kg)	13.98±0.570#
Pterostilbene (40 mg/kg)	10.56±0.501#
Metformin (500 mg/kg)	6.32±0.346#

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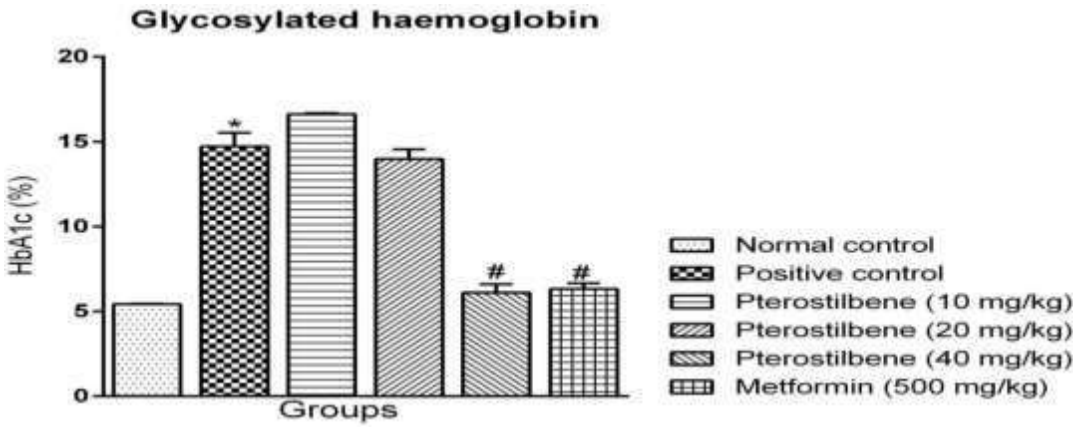
Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.



Impact of Pterostilbene on Glycosylated hemoglobin in Streptozotocin and HFD Induced diabetic rats.

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal

(6) Hemoglobin (g/dl):

Hemoglobin level in Positive control group was significantly decreased as compared to normal control group. Treatment with Pterostilbene at the doses of 10, 20 or 40 mg/kg and 500 mg/kg Metformin significantly improves the hemoglobin level when compared to the diabetic control group.

Impact of Pterostilbene on hemoglobin in Streptozotocin and HFD Induced diabetic rats.

Groups	**Hemoglobin (g/dl)**
Normal control	12.36±0.123
Positive control	9.31±0.205*
Pterostilbene (10 mg/kg)	10.30±0.031#
Pterostilbene (20 mg/kg)	10.98±0.056#
Pterostilbene (40 mg/kg)	11.76±0.156#
Metformin (500 mg/kg)	10.66±0.154#

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

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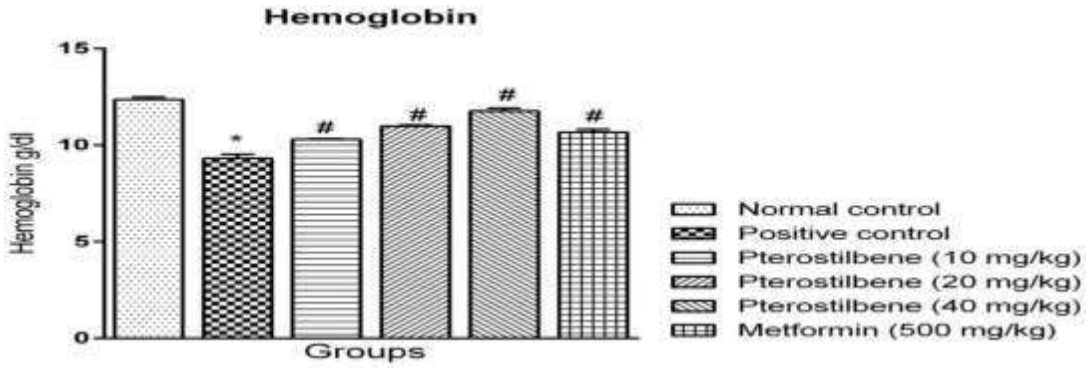


Impact of Pterostilbene on hemoglobin in Streptozotocin and HFD Induced diabetic rats.

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group.

#p <0.05 when compared to Positive control group.

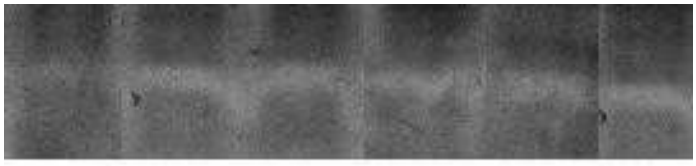
(7) MMP-9:

Positive control group exhibited significantly higher MMP-9 level as compared to Normal control group. Treatment with 10, 20 or 40 mg/kg Pterostilbene and 500 mg/kg Metformin significantly reduce the elevated MMP-9 levels.

Impact of Pterostilbene on MMP-9 levels in Streptozotocin and HFD Induced diabetic rats.

Groups	**MMP-9 levels**
Normal control	0.2668±0.005
Positive control	1.0000±0.001*
Pterostilbene (10 mg/kg)	0.9619±0.004#
Pterostilbene (20 mg/kg)	0.6762±0.004#
Pterostilbene (40 mg/kg)	0.5772±0.005#
Metformin (500 mg/kg)	0.6501±0.007#

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.



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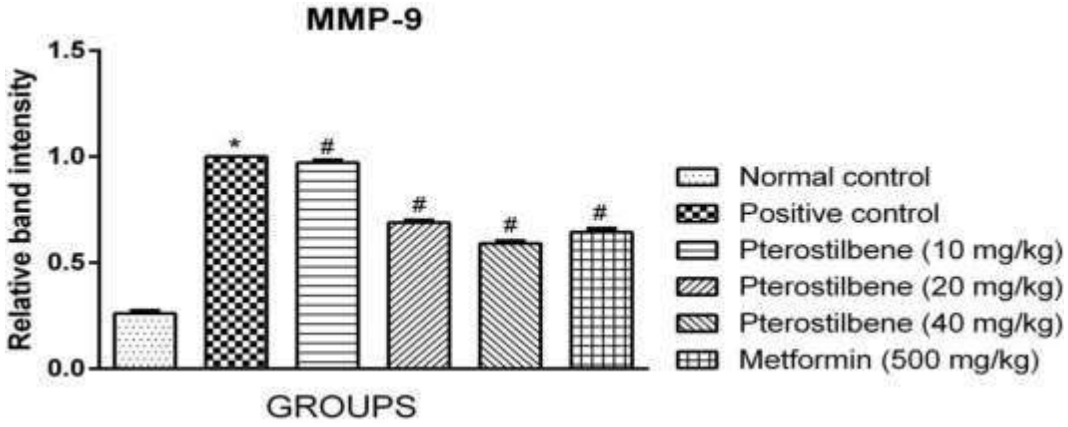
Gelatin zymography image of serum samples. (Please give me 2 more days for ladder)



Impact of Pterostilbene on MMP-9 levels in Streptozotocin and HFD Induced diabetic rats.

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

(8) INDICES OF INSULIN SENSITIVITY:

(8.1) HOMA-IR.

Positive control group exhibited significantly higher HOMA-IR level as compared to Normal control group. Treatment with 10, 20 or 40 mg/kg Pterostilbene and 500 mg/kg Metformin significantly reduce the elevated HOMA-IR levels.

Impact of Pterostilbene on HOMA-IR in Streptozotocin and HFD Induced diabetic rats.

Groups	**HOMA-IR**
Normal control	4.35±0.346
Positive control	7.32±0.095*
Pterostilbene (10 mg/kg)	6.92±0.332#
Pterostilbene (20 mg/kg)	6.14±0.201#
Pterostilbene (40 mg/kg)	4.85±0.091#
Metformin (500 mg/kg)	4.62±0.361#

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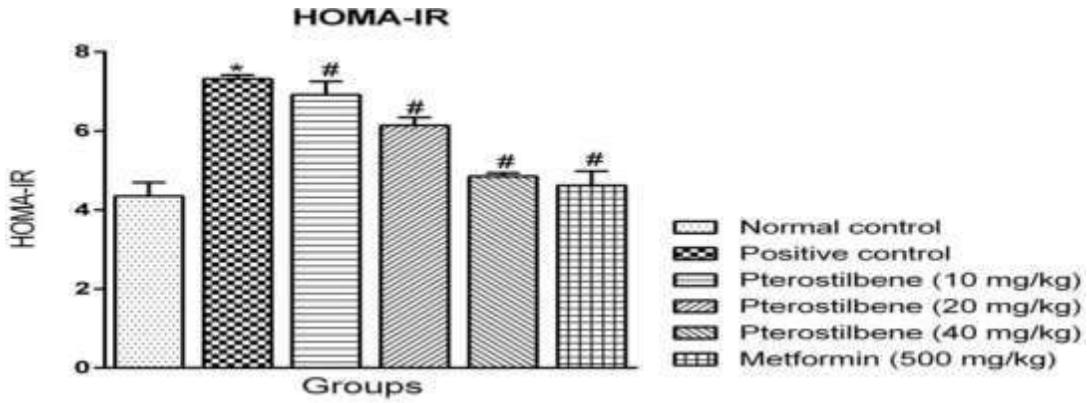
Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

Impact of Pterostilbene on HOMA-IR in Streptozotocin and HFD Induced diabetic rats.



Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's
post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

(8.2) HOMA-β:

Positive control group exhibited significantly lower HOMA-**β** level as compared to Normal control group. Treatment with 10, 20 or 40 mg/kg Pterostilbene and 500 mg/kg Metformin significantly elevate the reduced HOMA-**β** level.

Impact of Pterostilbene on HOMAβ- in Streptozotocin and HFD Induced diabetic rats.

Groups	**HOMA-β**
Normal control	124.94±3.33
Positive control	70.98±2.19*
Pterostilbene (10 mg/kg)	78.43±0.84#
Pterostilbene (20 mg/kg)	96.23±1.47#
Pterostilbene (40 mg/kg)	116.32±3.44#
Metformin (500 mg/kg)	118.35±2.97#

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

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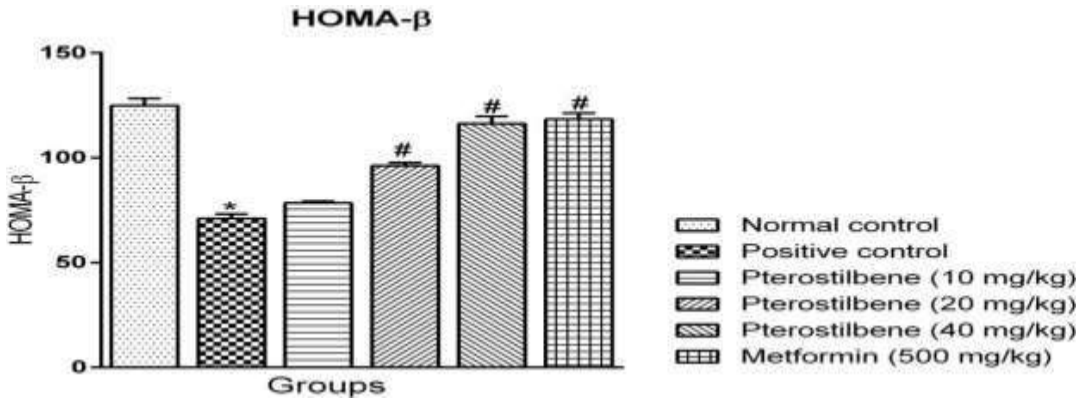

Impact of Pterostilbene on HOMA- β in Streptozotocin and HFD Induced diabetic Rats.
Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

(8.3) SERUM INSULIN (IU/ml):
Positive control group exhibited significantly lower serum insulin level as compared to Normal control group. Treatment with 10, 20 or 40 mg/kg Pterostilbene and 500 mg/kg Metformin significantly elevate the reduced serum insulin level.

Impact of Pterostilbene on serum insulin in Streptozotocin and HFD Induced diabetic rats.

Groups	Serum insulin (IU/ml)
Normal control	37.63±0.830
Positive control	18.04±0.570*
Pterostilbene (10 mg/kg)	20.56±0.790#
Pterostilbene (20 mg/kg)	24.15±0.800#
Pterostilbene (40 mg/kg)	32.06±0.870#
Metformin (500 mg/kg)	33.23±1.137#

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

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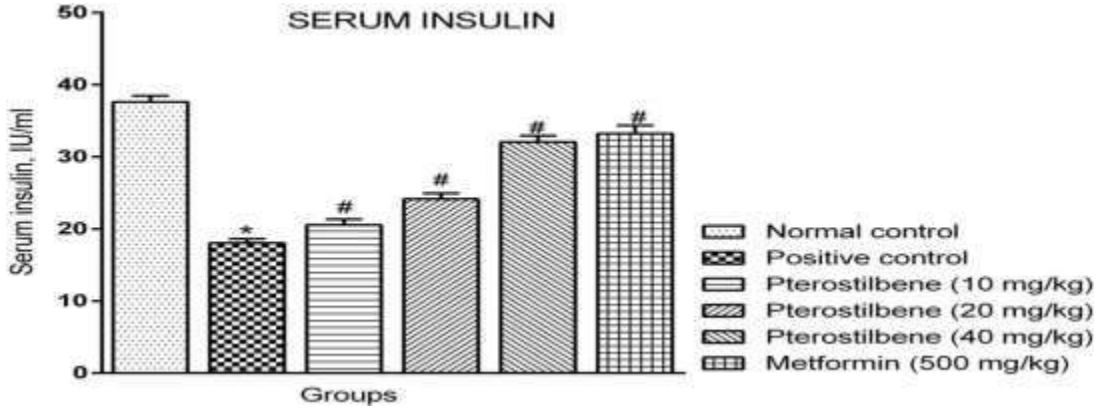

Impact of Pterostilbene on serum insulin in Streptozotocin and HFD Induced diabetic rats.

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

(9)Liver function test

(9.1) ALT (IU/L):

Positive control group exhibited significantly higher ALT as compared to those of Normal control group.

Treatment with 10, 20 or 40 mg/kg Pterostilbene and 500 mg/kg Metformin significantly reduce the elevated ALTlevels.

Impact of Pterostilbene on ALT in Streptozotocin and HFD Induced diabetic rats.

Groups	**ALT (IU/L)**
Normal control	47.152±1.174
Positive control	88.143±0.926*
Pterostilbene (10 mg/kg)	70.514±1.580#
Pterostilbene (20 mg/kg)	67.158±0.848#
Pterostilbene (40 mg/kg)	41.182±0.622#
Metformin (500 mg/kg)	42.364±0.452#

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

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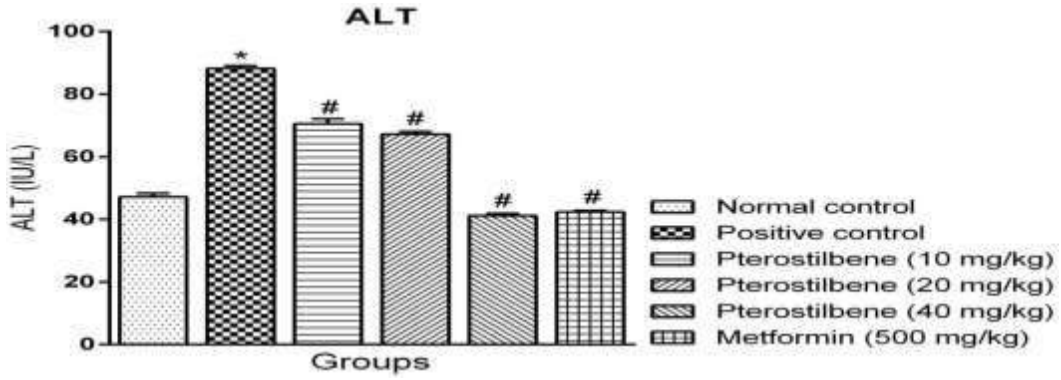

Impact of Pterostilbene on ALT in Streptozotocin and HFD Induced diabetic rats.

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's
post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

(9.2) AST (IU/L):

Positive control group exhibited significantly higher AST levels as compared to those of Normal control group. Treatment with 10, 20 or 40 mg/kg Pterostilbene and 500 mg/kg Metformin significantly reduce the elevated AST levels.

Impact of Pterostilbene on AST in Streptozotocin and HFD Induced diabetic rats.

Groups	AST (IU/L)
Normal control	84.22±1.60
Positive control	189.18±5.94*
Pterostilbene (10 mg/kg)	155.75±4.77
Pterostilbene (20 mg/kg)	127.51±4.03
Pterostilbene (40 mg/kg)	90.12±2.68
Metformin (500 mg/kg)	95.47±2.99

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's
post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

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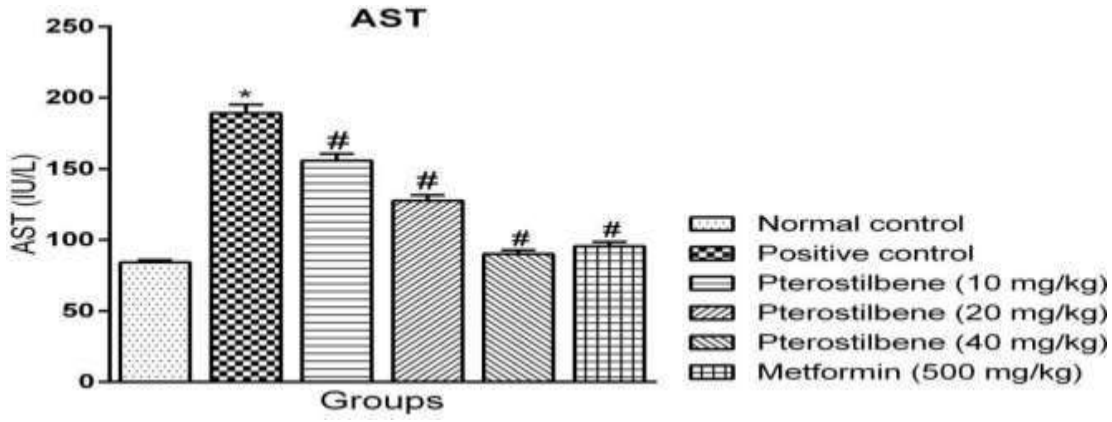

Impact of Pterostilbene on AST in Streptozotocin and HFD Induced diabetic rats.

Values are expressed as mean ± SEM (n=8), statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. *p <0.05 when compared to normal control group. #p <0.05 when compared to Positive control group.

III. STATISTICAL ANALYSIS:

All the values are expressed as Mean ± SEM (n=8). Statistical analysis was performed by of variance (ANOVA) followed by Tukey's test, comparison were made considering surgery, diet and treatment. OGTT was analyzed using two way ANOVA considering time and treatment as variables.

IV. RESULTS:

48 Rats were provided with fructose 10% solution in drinking water ad libitum for 14 days. Streptozotocin in citrate buffer pH 4.5 was injected intraperitoneally on day 15th. One week after the Streptozotocin injection, on the 21st day blood glucose level were measured. The rats with non fasting blood glucose more than 250 mg/dl were considered as diabetic and allocated randomly to all treatment groups. They were divided into 6 groups. The respective treatments were initiated from the 22nd day, each day till the 63rd day. On the 63rd day of treatment, overnight fasted rats shall be mildly anesthetized with ketamine (60 mg/kg) and xylazine (6 mg/kg) (10:1) combination intraperitoneally. 0.5 ml blood will be collected by retro-orbital route using alternative eyes at each of 0, 30, 60, 90,120 and 180 min time intervals for oral glucose tolerance test and estimating serum insulin. The animals were then euthanized using solid carbon dioxide chamber, blood will be collected from abdominal aorta to estimate various biochemical markers (lipid profile parameters, hemoglobin, MMP-9, antioxidant activity).

V. DISCUSSION:

Diabetes mellitus is a worldwide problem, and type 2 diabetis is found to be more prevalent (Home, 1998). In the present study, the effects of Pterostilbene, a resveratrol derivative which is metabolized to a lesser extent, have been assessed. It is well known that a high-fat, high-fructose diet (obesogenic diet), as we used in the present study, leads not only to increased body and fat accumulation, but also to insulin resistance. Basal glucose data in the present study show that, indeed, this alteration in glucose homeostasis was induced by the obesogenic diet. In the present study, Pterostilbene at a dose of 10, 20 or 40 mg kg, partially prevented insulin resistance, as shown by both HOMA-IR values and the glucose tolerance test. Indeed, data concerning these two parameters were significantly lower than those found in non-treated rats fed the obesogenic diet. In the 40 mg/kg dose of Pterostilbene, the glycemic control was ameliorated, as shown by the significant reduction in serum glucose levels and the oral glucose tolerance test. We can state that the improvement in glycemic control induced by pterostilbene was partial in the obesogenic diet fed animals. Pterostilbene at a dose of 40 mg per kg per day is efficient in lowering serum glucose levels than Metformin. It may be suggested that increased glucose utilization induced by this phenolic compound is on the basis of its anti-diabetic effect. These results partially agree with those reported by (Pari et.al 2006) in Streptozotocin–nicotinamide-treated rats, orally administered with 40 mg/kg of pterostilbene. The present study was aimed to investigate the impact of Pterostilbene in STZ and HFD induced diabetis in rats. It was observed that there was significantly increased in the fasting blood glucose (FBG), triglyceride (TG), total

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cholesterol (TC), serum biochemical parameters (SGOT, SGPT), blood glycosylated hemoglobin (HbA1c), MMP-9 levels, lipid peroxidation (MDA) and significantly decreased in the HDL, total protein, body weight and liver antioxidants enzymes (GSH, SOD, LPO CAT) levels in the Positive control group when compared to normal control group. The treatment with Pterostilbene at doses of 10, 20 or 40 mg/kg and Standard Metformin 500 mg/kg by orally continuous for 42 days significantly normalized elevated blood glucose level, glycosylated hemoglobin, body weight, MMP-9 levels and restored serum and liver biochemical parameters towards normal values. A reduced glucose tolerance was observed in Positive control group in the oral glucose tolerance test (OGTT). Oral administration of Pterostilbene significantly improved the impaired glucose tolerance in the glucose loaded rats. Considering the above result, the OGTT indicated an enhanced glucose utilization triggered by insulin production from the beta cells. It was also evident from the result that Pterostilbene significantly lowered FBG level in STZ- induced diabetic rats. Induction of diabetes with STZ is associated with a characteristic loss of body weight, which is due to increased muscle wasting and loss of tissue proteins (Swanston et al., 1990). Diabetic rats treated with Pterostilbene showed significant improvement in body weight. Hence Pterostilbene exhibited a marked effect in controlling the loss of body weight of diabetic rats. Streptozotocin along with HFD resulted in elevation of triglycerides, total cholesterol and decrease HDL cholesterol. Hypercholesteremia and hypertriglyceridemia are primary factors involved in the development of coronary heart disease which are the secondary complications of diabetes (Ananthan et al., 2003). Hyperlipidemia is characterized by high plasma levels of total cholesterol, LDL- cholesterol and triglycerides, with low plasma levels of HDL cholesterol. A variety of dysfunction in metabolic and regulatory mechanisms, due to insulin deficiency, is responsible for accumulation of lipids (Rajalingam et al., 1993). Pterostilbene significantly reduced serum triglycerides, total cholesterol and increase level of HDL in STZ induced diabetic rats. Streptozotocin mediated persistent hyperglycemia is a proximate cause of deterioration of β- cell function, which is mediated and complicated through the enhanced formation and generation of ROS (Kaneto et al., 2005 & Leung and Chan, 2009). From the results hyperglycemia was confirmed in the experimental animals by the significant elevation of blood glucose levels and glycosylated hemoglobin level as compared with the normal control rats. The amount of HbA1c increase is directly proportional to the fasting blood glucose levels. Administration of Pterostilbene to diabetic rats significantly reduced the glycosylated hemoglobin. As compared to Fasting Blood Glucose (FBG) and Oral Glucose Tolerance Test (OGTT), HbA1c is a better diagnostic parameter for diabetic complications. Elevation of serum biomarker enzymes such as SGOT, SGPT was observed in diabetic rats indicating impaired liver function, which was obviously due to hepatocellular necrosis. It has been reported that liver necrosis occurred in STZ-induced diabetic rats (Ohaeri, 2001). Therefore, increase in the activities of AST, ALT gives an indication on the hepatotoxic effect of STZ. Fourty two days of treatment with Pterostilbene reduced all the above mentioned serum hepatic biochemical parameters toward the normal values in a dose-dependent manner, thereby alleviating liver damage caused by STZ-induced diabetes. Lipid peroxidation (LPO) is usually measured in terms of thiobarbituric acid reactive substance (TBARS) i.e., Malondialdehyde (MDA), as a biomarker of oxidative stress (Venukumar and Latha, 2002). The reduction in liver antioxidant status during diabetes may be the result of counteraction against increased formation of lipid peroxides (Sabu and Kuttan, 2004). A marked increase in the concentration of TBARS in STZ-induced diabetic rats indicated enhanced lipid peroxidation leading to tissue injury and failure of the endogenous antioxidant defense mechanisms to prevent over production of free radicals. Treatment with Pterostilbene inhibited hepatic lipid peroxidation in diabetic rats as revealed by the reduction of TBARS levels towards normal control group, that suggesting Pterostilbene could improve the pathologic condition of diabetes by inhibiting lipid peroxidation in diabetic rats. It has been shown that in diabetic conditions, antioxidant defenses are altered due to the presence of chronic hyperglycemia thus promoting free radical regeneration ((Florence et al., 2013). The SOD, GSH, LPO and catalase are the four major antioxidant enzymes which play an important role in scavenging and that elimination of free radicals in the cells. The decrease in activity of these enzymes can lead to an excess availability of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) in the biological systems, which in turn generate hydroxyl radicals (OH), resulting in

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initiation and propagation of lipid peroxidation (Latha and Pari, 2003). SOD protects from oxygen free radicals by catalyzing the removal of superoxide radical, which damage the membrane and biological structures. Glutathione is tripeptide enzyme which plays an important role in the endogenous nonenzymatic antioxidant system. Primarily, it acts as reducing agent and detoxifies hydrogen peroxide in presence of an enzyme, glutathione peroxidase (Biswas et al., 2011). The depleted GSH may be due to reduction in GSH Synthesis or degradation of GSH by oxidative stress in STZ-induced hyperglycemic animals (Loven et al., 1986). Catalase was shown to be responsible for the detoxification of H2O2, and protects the tissues from highly reactive hydroxyl radicals (Mahboob et al., 2005). This decrease in catalase activity could result from inactivation by glycation of enzyme (Yan and Harding, 1997). In the present study, Pterostilbene treated groups showed a significant increase in the hepatic SOD, catalase and GSH activities in the diabetic rats. This means that the Pterostilbene can reduce the potential glycation of enzymes or theymay reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes. This result clearly shows that Pterostilbene contain a free radical scavenging activity, which could exert a beneficial action against pathological alteration caused by the presence of superoxide radicals and hydrogen peroxide radical. In addition to the release of oxidants, the activated cells also spill out protease enzymes MMP-9 which cause break down of connective tissue component. Higher MMP-9 levels have been found in Diabetes Mellitus Type-2 Patients (Sudip Das et al., 2013). This study shows that pterostilbene produce a marked decrease in blood glucose at 40 mg/kg body weight in diabetic rats after 9 weeks of treatment. Our findings were in agreement with those reported by Manickamet al (1997). The antidiabetic effect of pterostilbene may be due to increased release of insulin from the existing β- cells of pancreas. It is well established that Metformin reduce fasting plasma glucose concentration by reducing rates of hepatic glucose production (Bailey and Turner, 1996; Cusi and Defronzo, 1998), its effect on the relative contribution of hepatic glycogenolysis (Cusi et al., 1996; Christiansen et al, 1997) and gluconeogenesis (Stumvoll et al., 1995). In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including hemoglobin (Alberti and Press, 1982). Glycosylated hemoglobin (HbA1c) was significantly increased in diabetic animals, and this increase was found directly proportional to the fasting blood glucose level (Koenig et al., 1976). During diabetes, the excess glucose present in blood reacts with hemoglobin. Therefore, the total hemoglobin level is decreased in diabetic rats (Sheela and Augusti, 1992). Administration of pterostilbene prevents a significant elevation in glycosylated hemoglobin thereby increasing the level of total hemoglobin in diabetic rats. The antihyperglycemic activity of Pterostilbene observed previously was through enhancement in peripheral utilization of glucose and consequently restores the deficient hepatic glycogen content in Streptozotocin-induced model (Grover et al., 2002). In our rat model, STZ and HFD exposures showed increased MMP-9 activity in the Serum samples assessed by gelatin zymography, as compared to normal control group. Increased MMP-9 activity was indicated by intense gelatinolytic bands on gelatin containing polyacrylamide gels. Such augmented MMP-9 activitywas found to be affected by treatment with Pterostilbene 40 mg/kg & Metformin 500 mg/kg and shows decrease in MMP-9 activity, although the decrease was not found to be statistically significant with 20 and 40 mg/kg of Pterostilbene. On the basis of all the evidence it is possible that these activities are due to Pterostilbene. Therefore, it can be concluded that Pterostilbene remarkably effective against Streptozotocin-induced diabetes in SD rats plausibly by virtue of its augmenting the endogenous antioxidant mechanisms and MMP-9 levels in diabetic complications. The present study found that pterostilbene as the main constituent of Pterocarpus marsupium might contribute to its antidiabetic action. We conclude that pterostilbene has beneficial effects on glucose concentration as well as sequential metabolic correlation between increased glycolysis and decreased gluconeogenesis stimulated by pterostilbene suggests the possible biochemical mechanism through which glucose homeostasis are regulated.

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