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HEDERAGENIN AMELIORATES ANTIDIABETIC, ANTIHYPERLIPIDEMIC AND ANTIOXIDANT EFFECTS IN HIGH FAT DIET / STREPTOZOTOCIN-INDUCED DIABETES IN MICE

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ABSTRACT

The present study describes the antidiabetic effect of the hederagenin from Sapindus *trifolitis* inhibited dipeptidyl peptidase IV (DPP-IV) *in vitro* with an IC_{50} of 110.27±12.9 µg/ml. Hederagenin at doses of 200, 400 and 600 mg/kg p.o., also produced dose-dependent mean percent reductions of glucose excursion ($AUC_{0–120\ min}$) respectively in lean mice. However, even the highest dose of hederagenin did not alter normoglycemic condition. Hederagenin at dose of 200 mg/kg/day, p.o.for 28 days produced significant ($p < 0.05$) reduction in bodyweight, plasma glucose (PG), triglycerides (TG) total cholesterol (TC), BUN (Blood Urea Nitrogen) and CREA(creatinine) content in high-fat streptozotocin-induced diabetic mice. Hederagenin also improved oral glucose tolerance significantly ($p < 0.05$) reduced glucose excursion ($AUC_{0–120}$ min) and significantly ($p < 0.05$) enhanced the endogenous antioxidant status when compared to diabetic control. Hederagenin preserved islet architecture and prevented hypertrophy of Bowman's capsule as evident from the histopathology of pancreas and kidney. Hederagenin did not show any detectable haematological toxicity at therapeutic doses. In conclusion, Hederagenin exhibits antidiabetic effect possibly by inhibiting DPP-IV and improving antioxidant levels in high fat diet/ streptozotocin (HFD/STZ) diabetic mice.

KEYWORD: Sapindus *trifolitis*, hederagenin, antidiabetic, streptozotocin.

1. INTRODUCTION

Diabetes mellitus (DM) in humans is a manifestation of metabolic disturbances due to the dietary intake of excess carbohydrates and lipids.[1] Hyperglycemia and hyperlipidemia are important risk factors in the development of cardiovascular disease and metabolic disorders.[2,3] A worldwide survey reported that the estimated incidence of diabetes and projection for year 2030 is going to be 350 million.[4,5] The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered.[6,7] Various chemicals have been used to induce diabetes in rodents, particularly streptozotocin (STZ), which has been extensively used in diabetes research. The development of hyperglycemia, following STZ injection is primarily due to the direct pancreatic β-cell destruction, and resulting insulin deficiency.[8,9] There is a correlation between diabetes and high fat diet (HFD) observed in rodents. In similar lines, mice fed with HFD and injected with STZ, become significantly hyperglycemic, hyperlipidemic and develop weight gain.[10,11] Currently, the antidiabetic drugs in use for long term therapy are found to be associated with various toxicities owing to which the developmental process in antidiabetic drug discovery has shifted its focus to natural plant sources having minimal side effects.[12,13] Plants play a major role in the discovery of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemic and hypolipidemic agents.[14] Hederagenin are being used to treat diabetes and dyslipidemia.[15] This is based on the fact that, excessive oxidative stress is implicated in the pathology and complications of DM and Hederagenin with antioxidant properties exert beneficial antidiabetic effect by correcting the disturbed oxidative milieu in diabetic conditions.[16,17] Between 2001 and 2012, many new drugs derived from natural products were introduced for the treatment of dyslipidemia and diabetes.[18]

Sapindus trifoliatus (L.), (family: Sapindaceae), fruit carp is claimed to possess medicinal property such emetic activity stomachic, antihelminthic and is traditionally used in treatment of asthma, inflammation and also used for epilepsy The constituents, such as hederagenin glycosides (3beta,4alpha)-3,23-Dihydroxyolean-12-en-28-oic acid), Acyclic sesquiterpene Oligoglycosides and Sapindic acid, have been isolated from this plant as.[19] Hederagenin shows significant biological effect in inflammation, cancer and antinociceptive acitivity[15] demonstrated that hederagenin bisdesmoside could be converted to its monodesmosides and its serial saponins by human intestinal bacteria and that these metabolites showed anti-diabetic activity. Thus, the aim of the present study was to analyze the activity of hederagenin

in the glucose lowering effect in a high-fat diet and streptozotocin induced murine model of diabetes.

2. MATERIAL AND METHODS

2.1. Chemicals

Dipeptidyl peptidase IV (DPP-IV) from porcine kidney, quercetin and glycine-proline-pNA (Gly-Pro-pNA), streptozotocin (STZ), catalase, superoxide dismutase (SOD) was purchased from Sigma–Aldrich, St. Louis, MO, USA. BCA protein kit was procured from Thermo Fisher Scientific Inc., MA, USA.

2.2. Animals

Male Swiss albino mice weighing 28–30 g were purchased from Sasthra college of Pharmacy, Nellore, India. All mice were housed 2/cage and fed standard laboratory chow in the animal room with 12 h dark/light cycles and constant temperature of 20 ± 5^{o}C. All the studies were conducted in compliance with guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and approved by the Institutional Ethical Committee (IAEC) Protocol no 12/02.

2.3. Extraction and preparation of hederagenin

Fruits of *Sapindus trifoliatus* (L.), (family: Sapindaceae) were collected from PERD Centre, Ahmedabad, and Gujarat, India. The plant was authenticated by comparing with the herbarium voucher specimen deposited at Presidency College Madras University Herbarium (Specimen No. PBPB-41). The material was air dried in shade, powdered mechanically and stored in airtight containers. The extract was filtered, pooled and the solvent was removed under reduced pressure at 50 ± 60°C using rotary flash evaporator and the yield was 7.6g, 0.76%w/w of hederagenin.

2.3. Isolation

The Coarse powder of dry fruits of *Sapindus trifoliatus* was extracted exhaustively with 8% methanolic sulphuric acid and the extract so obtained was chromatographed on silica gel column and eluted successively using chloroform and methanol in the ratio of 1:1. The eluted fractions were collected at an interval of 5ml each and were monitored by thin layer chromatography and grouped into five fractions. The fraction two recovered in higher concentration was recrystallized from chloroform to get an colorless crystals. The structure was confirmed by IR, 1H NMR and mass spectral studies.

2.4. *In vitro* DPP-IV enzyme activity

DPP-IV enzyme cleaves the chromogenic protein Gly-Pro-p-NA at a position next to proline residue to give a yellow chromogen, p-nitroaniline, resulting to an increase in absorbance at 405 nm. Briefly, the assay was conducted by adding 10 µl DPP-IV enzyme in 100 µl of the assay buffer in 96-well plates. The mixture was incubated for 30min at 37C after addition of 10 µl of hederagenin was initiated by adding 80µl of Gly-Pro-pNA (500µM), incubated at 37^{o} C for 30 min. The absorbance was measured at 405 nm using microplate reader (ELx800, Bio-Tek Instruments, USA).[13] Percentage inhibition was calculated as,

$$\textbf{\% Inhibition} = \frac{\text{Absorbance}_{Control} - \text{Absorbance}_{test}}{\text{Absorbance}_{Control}} \times 100$$

2.5. Oral glucose tolerance test (OGTT) in lean mice

The animals were fasted overnight before OGTT and basal blood samples (T_0) were taken. Distilled water (normal control) or glucose load of 2 g/kg, p.o. were administered immediately after treatment with 0.25% w/v (10 ml/kg) carboxymethyl cellulose (CMC) (vehicle control) or metformin (500 mg/kg, p.o.), Hederagenin (200, 400 and 600 mg/kg, p.o.) respectively. Blood samples were collected by tail cut method at different time points (T_{15}–T_{120} min : 15, 30, 60 and120 min) after glucose administration and blood glucose was measured by using commercially available glucometer (Accu-Check, Roche, Germany).The reduction in blood glucose produced by metformin and Hederagenin was calculated using area under the curve ($AUC_{0–120}$ min).

2.6. Effects of hederagenin on plasma DPP-IV enzymatic activity in lean mice

Animals were fasted overnight and divided into three groups (n = 6). Following which they received different dose of hederagenin (200, 400 and 600 mg/kg, p.o). Blood samples were collected from retro-orbital plexus underlight anaesthesia at 0, 15, 30, 60, 120 min post treatment. Plasma was separated and DPP-IV enzyme activity was analyzed according to method described earlier.[13]

2.7. Effects of hederagenin on fasting blood glucose levels in normoglycemic mice

Animals were fasted overnight, divided into three groups (n=6) and basal blood samples (T_0) were taken. Following which they received a 0.25% w/v CMC or glibenclamide (10mg/kg, p.o.) or highest tested dose of hederagenin (600mg/kg, p.o.). Blood samples were collected by tail cut method at different time points (T_{15}–T_{120} min: 15, 30, 60 and 120 min) post treatment and blood

glucose was measured by using commercially available glucometer (Accu-Check, Roche, Germany).The results were expressed as area under the curve ($AUC_{0–120\ min}$).[20]

2.8. Effects of hederagenin on high fat diet/streptozotocin (HFD/STZ)-induced diabetic mice

Four week-old male animals were fed with HFD(~50% kcal) comprising of components providing different percentage of energy.[10] The detailed composition of HFD is given below (Table 1). After 4 weeks of HFD feeding, the mice were injected once with low-dose STZ (125mg/kg,i.p.) to induce partial insulin deficiency. Two weeks after STZ injection, the majority of HFD/STZ-treated mice displayed hyperglycemia and glucose intolerance. At six weeks of HFD feeding, animals with similar degrees of hyperglycemia and body weight were randomly divided to control or treatment groups. The diabetic control and the normal control groups received the vehicle (0.25% CMC, 10ml/kg) and the treatment groups were given pioglitazone (10mg/kg, p.o.) and hederagenin (200 mg/kg, p.o) as suspensions in 0.25% CMC (10ml/kg), once daily. In the treatment schedule of 28 days, blood was collected on the 28th day by retro-orbital puncture under mild anesthesia, centrifuged and examined for PG, TC and TG. Subsequently, OGTT was performed at the end of the experiment. Body weight and daily food intakes were monitored. Animals were sacrificed and livers were isolated to measure antioxidant status intake were monitored.

2.8. Histopathology of mouse pancreas and kidney

The isolated pancreas and kidney tissues fixed in 10% neutral-buffered formalin, dehydrated by passing through a graded series of alcohol, and embedded in paraffin blocks and 5µm sections were prepared using a semi-automated rotary microtome (model RM2245, Leica Microsystems, Wetzlar, Germany). The sections were stained in hematoxylin and eosin. The sections were mounted by Disterene Phthalate Xylene (D.P.X.).[21]

2.9. Biochemical analysis Plasma

Glucose, total cholesterol, triglycerides blood urea nitrogen and creatinine were estimated spectrometrically using standard enzymatic kits (Aspen Laboratories Pvt. Ltd., Delhi, India).Total protein was estimated by the BCA Protein Assay Kit (Thermo Fisher Scientific Inc., MA, USA). Lipid peroxidation was measured as malondialdehyde (DA).[22] Glutathione (GSH),[23] catalase (CAT)[24] and superoxide dismutase (SOD)[25] were determined following the standard protocols.

2.10. Effect of hederagenin on hematological analysis

To further evaluate the toxicological effect of chronic supplementation of hederagenin, hematological parameters such as white blood cell (WBC), red blood cell (RBC), platelet count, hemoglobin (Hb) level, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) were determined by the use of automated hematological analyzer (ADVIA-120,Siemens,India).

2.11. Statistical analysis

Statistical comparisons were performed using Graph Pad Prism 5.02 by one-way analysis of variance (ANOVA), followed by posthoc Tukey's test. Results were expressed as mean ± S.E.M. and $p < 0.05$ was considered significant.

3. RESULTS

3.1. *In vitro* DPP-IV inhibition by Hederagenin

Hederagenin exhibited dose dependent DPP-IV enzyme inhibition. IC_{50} hederagenin exhibited dose dependent DPP-IV enzyme inhibition. IC_{50} values of hederagenin and quercetinare 110.27±12.9 and 59.7±0.9µg/ml, respectively (Fig. 2).

3.2. Oral glucose tolerance test in lean mice

The acute effect of hederagenin was evaluated by OGTT on overnight fasted animals. Hederagenin significantly ($p < 0.05$) reduced blood glucose excursion by 35.4 % and 52.0 % at tested doses of 400 mg/kg and 600mg/kg respectively as shown by $AUC_{0–120}$ min (Fig. 3A). Results suggest improvement in glucose tolerance. Metformin also (500mg/kg) significantly ($p < 0.05$) lowered glucose excursion ($AUC_{0–120\ min}$) by 62.1 % as opposed to 52.0 % for hederagenin (600mg/kg) (Fig. 3B). However, hederagenin at 200 mg/kg did not exhibit any significant blood glucose lowering when compared with vehicle control.

3.3. Effect of PM1 on plasma DPP-IV activity

Plasma DPP-IV was inhibited in a dose dependent manner at 15 min post treatment and continued up to 2 hours of study (Fig. 4). The degree of improvement in glycemic control observed in the hederagenin treated animals was dependent on the extent of plasma DPP-IV inhibition.

3.4. Effects of Hederagenin on fasting blood glucose levels in normoglycemic mice

The effect of hederagenin (600mg/kg) on fasting blood glucose levels in normoglycemic mice was determined. Hederagenin did not affect fasting blood glucose level and maintained normoglycemic condition throughout the study(Fig. 5A and B).On the other hand, glibenclamide (10mg/kg) significantly ($p < 0.05$) reduced normal blood glucose levels.

3.5. Effects of Hederagenin on metabolic parameters in HFD/STZ-induced diabetic mice

We studied the chronic effects of hederagenin (200mg/kg) and pioglitazone (10mg/kg) on metabolic markers in HFD/STZ mice. The body weight of hederagenin treated animals was significantly ($p < 0.05$) lower than diabetic controls. However, pioglitazone did not produce any significant change in body weight (Table 3). To further evaluate the antidiabetic effect of hederagenin, we estimated the metabolic markers associated with DM (PG, TG, TC, BUN and CREA) in the different groups.

Both Hederagenin (200mg/kg) and pioglitazone (10mg/kg), significantly ($p < 0.05$) reduced compared to diabetic control (Table 2).

3.6. Effects of Hederagenin on OGTT in HFD/STZ-induced diabetic mice

The chronic effect of hederagenin was evaluated by OGTT on overnight fasted animals (Fig. 6A). Hederagenin at a dose of 200 mg/kg for 28days produced significant ($p < 0.05$) reduction in blood glucose excursion as shown by $AUC_{0–120}$ min. Both hederagenin (200mg/kg) and pioglitazone (10 mg/kg) significantly ($p < 0.05$) lowered glucose excursion by 52.0% and 43.7 % in $AUC_{0–120\ min}$ compared with diabetic control (Fig. 6B).

3.7. Effects of Hederagenin on liver antioxidant activity in HFD/STZ induced diabetic mice

Chronic supplementation with hederagenin and pioglitazone for 28 days resulted in significant ($p < 0.05$) elevation in liver antioxidants (GSH, CAT and SOD) when compared to diabetic control. Both hederagenin and pioglitazone significantly ($p < 0.05$) prevented membrane damage by decreasing lipid peroxidation compared to

diabetic control (Table 3).

3.8. Effect of Hederagenin on pancreas and liver histology in HFD/STZ diabetic mice

Normal control showed typical histological structure of pancreas with normal sized islets (Fig. 7A) whereas diabetic control showed shrinkage in size, decrease in number of the islets and destruction of β-cells (Fig. 7B) (26). Pioglitazone and hederagenin (Fig. 7C and D) treated mouse preserved islet cell architecture with minimal pathological changes.

In kidney of diabetic rats, modest glomerular lesions were noted with irregular glomerular capillaries, widened and attached to the Bowman's capsule (27). The above changes were decreased significantly in Pioglitazone and hederagenin group.

3.9. Effect of Hederagenin on hematological parameters

Chronic administration of hederagenin (200mg/kg), for 28days, did not alter the hematological parameters. RBC count, WBC count, platelet count, hemoglobin, % hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of all the hederagenin treated animals were within the normal range (Table 4).

Table 1

Composition of diet used in the experiment.	
Ingredients	**HFD(g/kg)**
Lard	270
Coconut oil	30
Cholesterol	10
Nutrilab diet	400
Casein	230
Cellulose	35
Vitamin mix	5
Mineral mix	5
Choline bitartrate	1
L-Cysteine	2.5
DL-Methionine	2.5

Table 2: Effect of Hederagenin on metabolic parameters in HFD/STZ mice.

Metabolic parameter				
	Normal Control	**Diabetic control**	**Pioglitazone (10mg/kg)**	**Hederagenin (200mg/kg)**
Final bodyweight(g)	28.9 ± 1.4	40.0 ± 0.61*	37 ± 0.8*	34.2 ± 1.0*,#
PG (mg/dl)	100.2 ± 8.4	241.6 ± 11.1*	137.6 ± 19.6*,#	90.2 ± 6.4 #
TG (mg/dl)	132.2 ± 6.4	229.6 ± 12.7*	143.5 ± 12.5*,#	116.8 ± 9.3 #
TC (mg/dl)	134.2 ± 12.6	203.6 ± 15.9*	197 ± 4.4*,#	134.8 ± 6.8 #
BUN (mg/dl)	30.01±1.93	231.76± 9.01*	94.23±3.2*,#	134.2±3.23*,#
CREA (mg/dl)	0.536±0.13	3.312± 0.08*	1.2254±0.26*,#	2.0129±*,#

Results are expressed as mean±S.E.M. PG, plasma glucose; TG,triglyceride; TC,total cholesterol; BUN, Blood urea nitrogen; CREA Creatinine.

Table 3: Effect of Hederagenin on antioxidant activity in HFD/STZ mice.

Antioxidant analysis				
	Normal Control	**Diabetic control**	**Pioglitazone (10mg/kg)**	**Hederagenin (200mg/kg)**
GSH (nmole/mg of protein)	29.2 ± 3.0	18.4 ± 1.1*	25.0 ± 1.4#	27.7 ± 3.6 #
CAT (U/mg of protein)	128.3 ± 7.4	71.2 ± 5.6*	113.2 ± 1.2#	131.3 ± 2.9 #
SOD (U/mg of protein)	213.2 ± 4.3	69.5 ± 9.3*	155.2 ± 6.3*,#	179.2 ± 12.3*,#
MDA (nmole/mg of protein)	0.29 ± 0.02	0.59 ± 0.01*	0.33 ± 0.03#	0.37 ± 0.02 #

Results are expressed as mean±S.E.M.

GSH glutathione; CAT, catalase; SOD, superoxide dismutase; MDA, malondialdehyde.

Table 4: Effect of Hederagenin on haematological parameters in mice.

Hematological parameters		
	Normal control	**Hederagenin(200mg/kg)**
RBC (10^3 µl)	7.9 ± 0.1	8.3 ± 0.1
WBC (10^6 µl)	13.9 ± 0.3	14.0 ± 1.0
Platelet (10^3 µl)	816 ± 12.5	893.43 ±22.3
Hb (g/dl)	14.3 ± 0.32	14.9 ± 1.0
% Hematocrit	26.7 ± 1.1	24.6 ± 1.1
MCV (fL)	41.5 ± 0.4	42.7 ± 0.5
MCH (pg)	19.2 ± 0.7	20.3 ± 0.2
MCHC (g/dL)	29.1 ± 0.5	29.5 ± 1.2

Results are expressed as mean ± S.E.M.

MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular haemoglobin concentration.

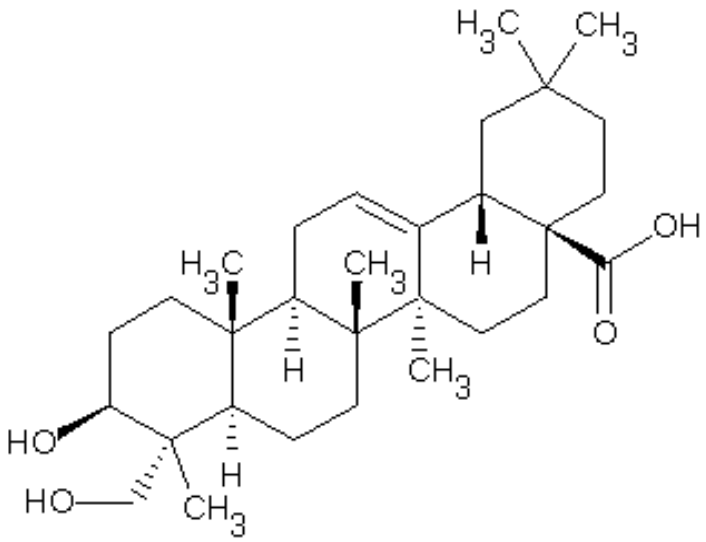


Figure.1. Structure of HEDERAGENIN

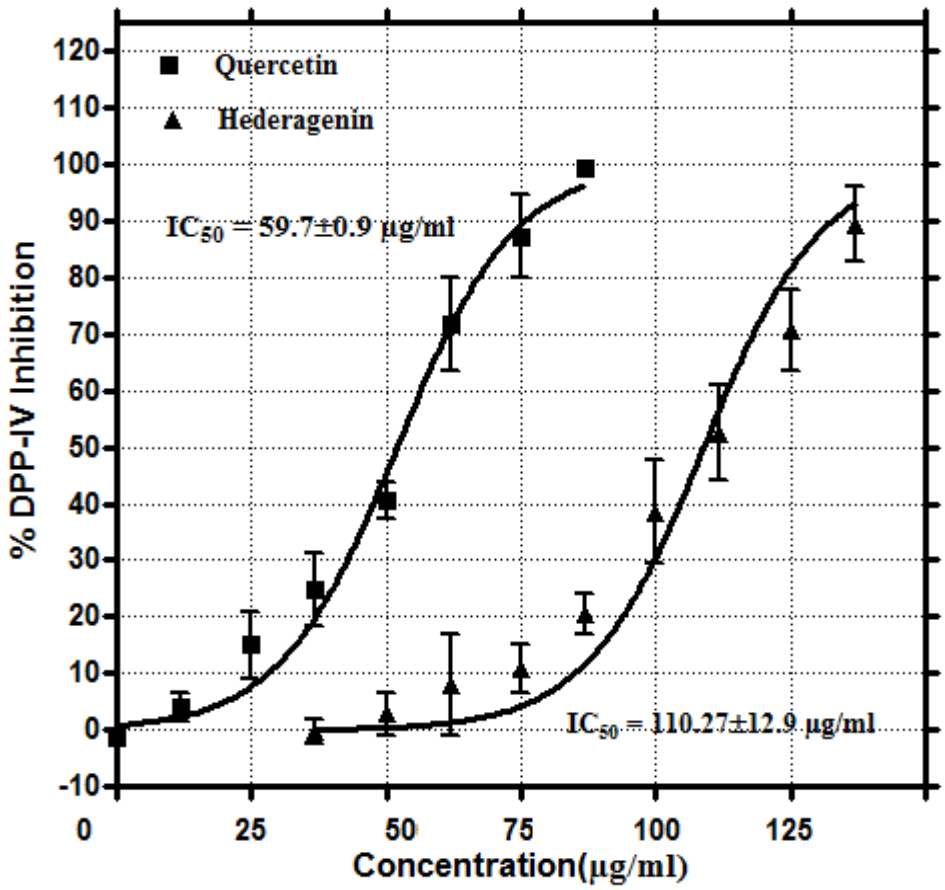


Figure. 2. In vitro DPP-IV inhibition activity of Hederagenin. Results are expressed as mean of triplicate measurements ± standard error.

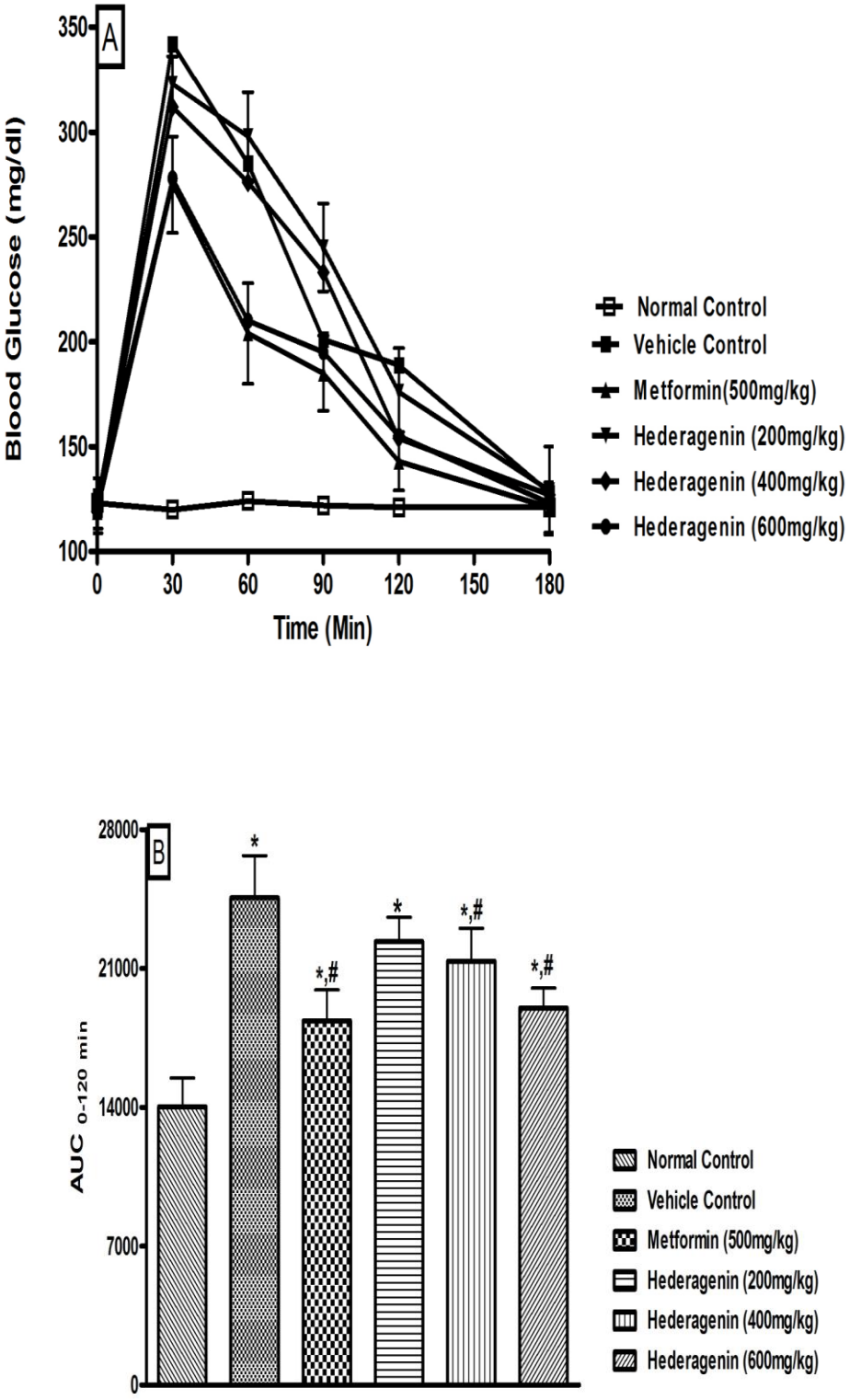


Figure.3. Effect of Hederagenin on OGTT in lean mice (A) blood glucose levels and (B) AUC $_{0–120\ min}$. Results are expressed as mean ±S.E.M. *p < 0.05 as compared to normal control and #p < 0.05 as compared to vehicle control

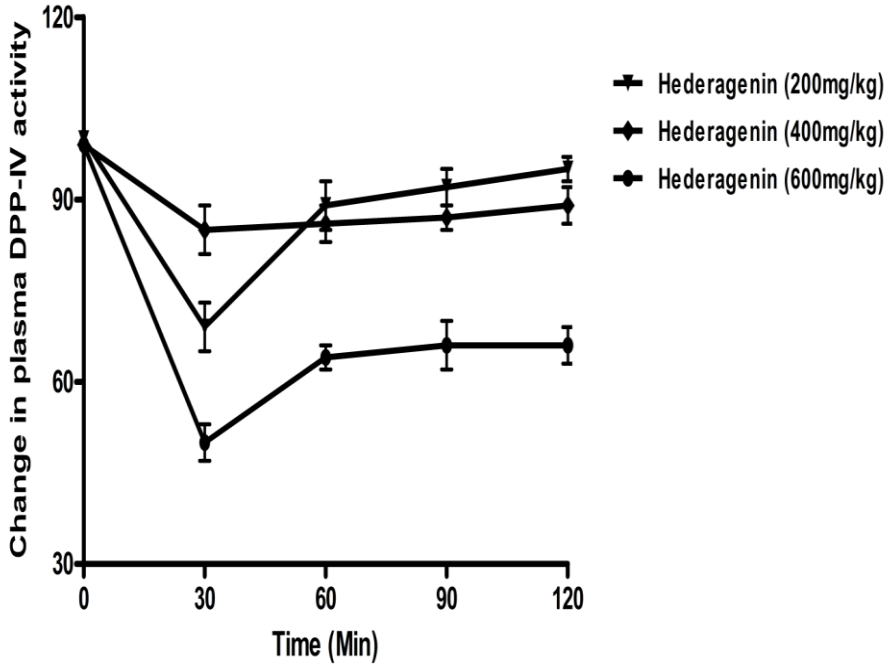
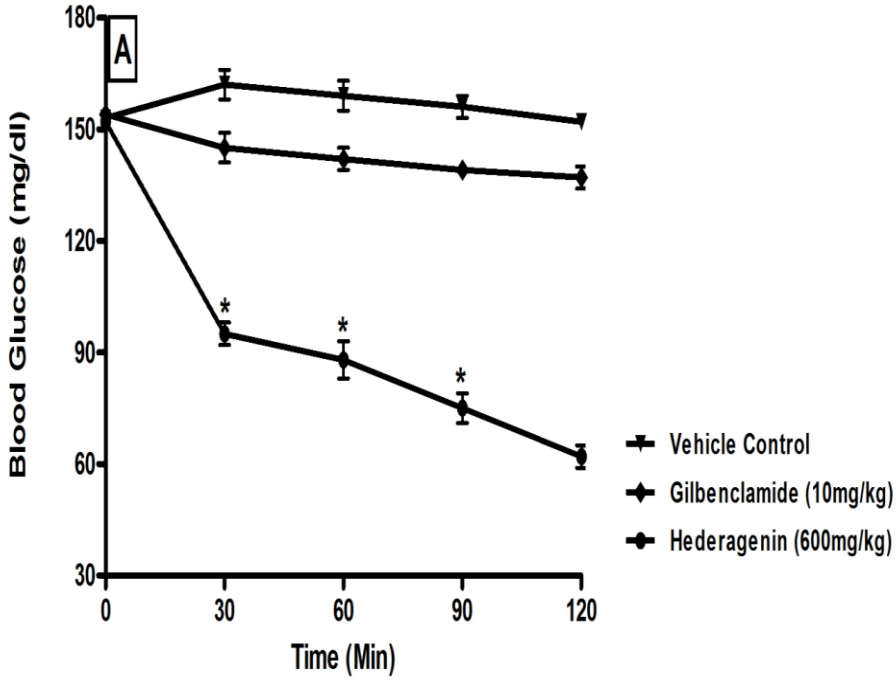


Figure.4. Acute inhibition by Hederagenin on plasma DPP-IV in lean mice. Results are expressed mean ±S.E.M.



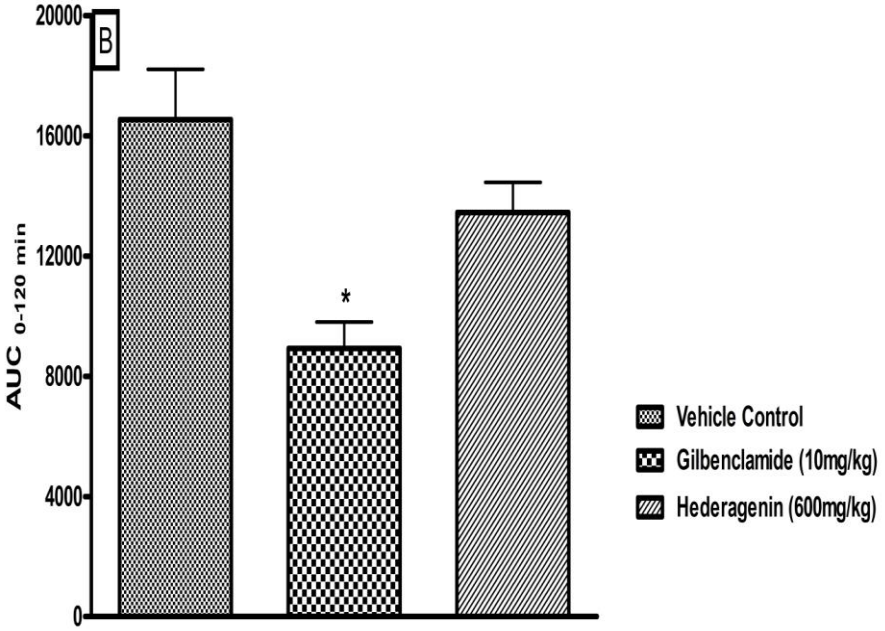
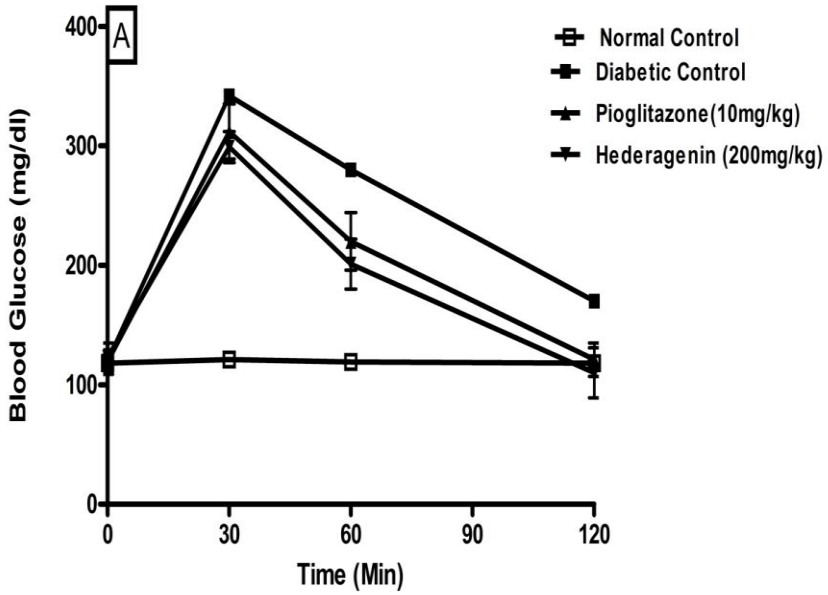


Figure.5. Effect of Hederagenin on blood glucose levels in normoglycemic mice (A) blood glucose levels and (B)$AUC_{0–120\ min}$. Results are expressed as mean ± S.E.M. *p < 0.05 as compared to vehicle control.



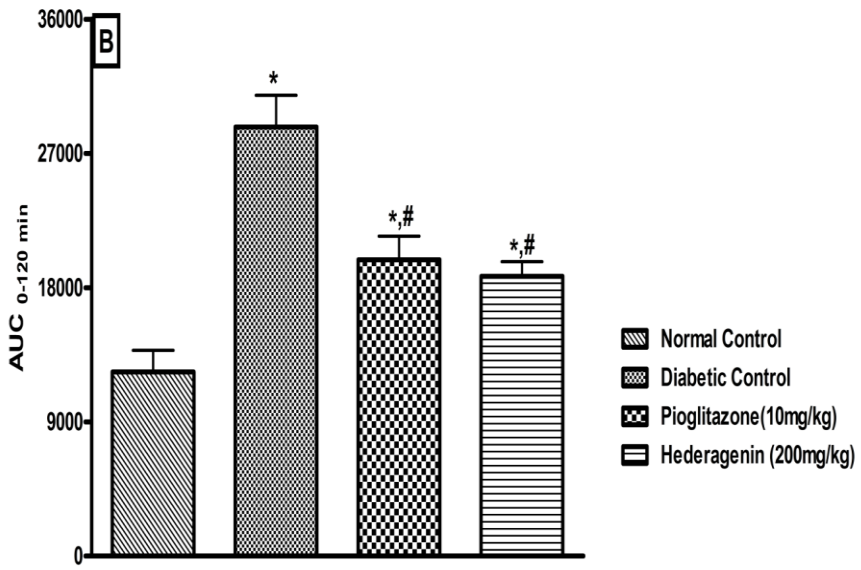
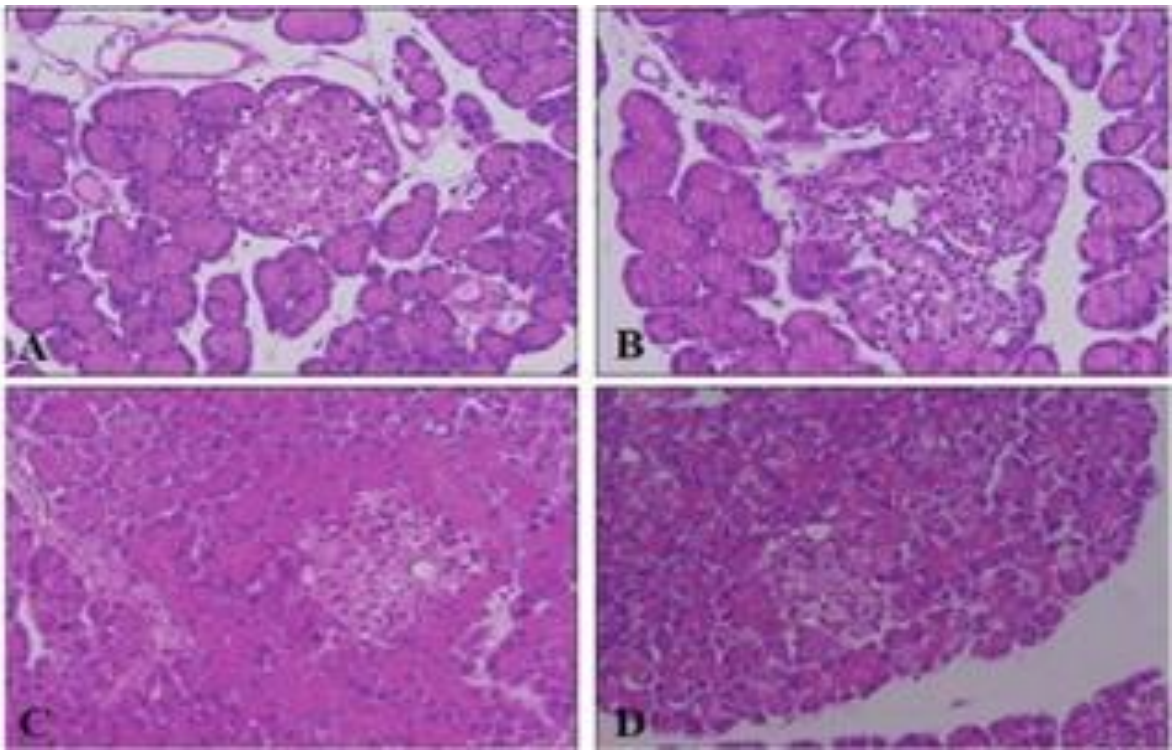
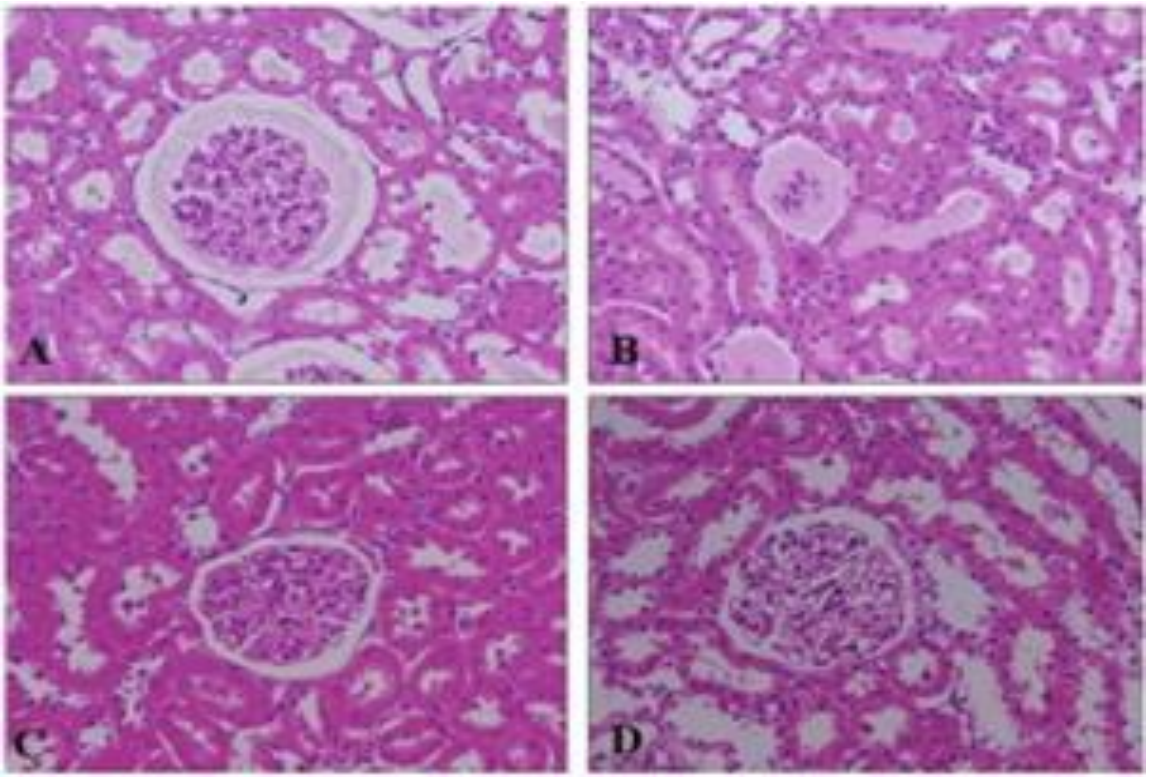


Figure.6.Effect of Hederagenin on OGTT in HFD/STZ mice (A) blood glucose levels and (B)$AUC_{0–120\ min}$. Results are expressed as mean ± S.E.M. *p < 0.05 as compared to normal control and # p<0.05 as compared to diabetic control.



7.A. Figure 1: Effect of hederagenin on histological changes of pancreatic islets at magnification of x200 . Group I (Normal control ;A), Group II (diabetic; B), Group III (pioglitazone (10 mg/kg,p.o.); C), and Group IV (Hederegenin ;D)



7.B. Figure 1: Effect of hederagenin on histological changes of Kidney at magnification of x200 . Group I (Normal control ;A), Group II (diabetic; B), Group III (pioglitazone (10 mg/kg,p.o.); C), and Group IV (Hederegenin ;D)

4.0. DISCUSSION

Evaluation of plant products in the treatment of DM is becoming profitable owing to the presence of several bioactive constituents with therapeutic potential. In recent years, several researchers have studied the efficacy of different medicinal plants in modulating the disturbed redox status associated with DM. Therefore, the present study was aimed at assessing the effect of hederagenin on hyperglycemia, lipid profile, enzymatic and non-enzymatic antioxidants in HFD/STZ-induced diabetic mice.

Drugs that inhibit DPP-IV enzyme have been demonstrated to decrease hyperglycemia and improve impaired glucose metabolism and promote insulin secretion from pancreatic β-cells.[10] The results of *in vitro* studies showed that hederagenin inhibited the DPP-IV enzyme dose-dependently. OGTT in lean mouse showed that hederagenin (400 and 600 mg / kg) significantly reduced glucose excursion ($AUC_{0–120}$ min) in a dose dependent manner, implying DPP-IV inhibition. Hederagenin appears to inhibit DPP-IV enzyme in both, time and dose-dependent manner, which was further confirmed by estimating DPP-IV in plasma of hederagenin treated mice. However, hederagenin, even at the highest dose (unlike

glibenclamide) did not reduce fasting blood glucose, suggesting only a corrective role, which is in sharp contrast with glibenclamide. Hypoglycemia is the most frequent adverse drug reaction associated with sulfonylureas and the uniformly euglycemic effect of hederagenin marks a significant advantage.

DM is increasing an alarming rate worldwide, including industry and developing countries, which can be mainly attributed to the sedentary life style and calorie rich diet. This in turn has been one of the major reasons for the increase in the incidence and prevalence of hyperlipidemia. DM is often linked with abnormal lipid metabolism and is considered a major risk factor for the premature development of atherosclerosis and cardiovascular complications.[28, 29] Implicit in this study, HFD-fed mice which are already mildly hyperglycemic, become more susceptible to develop significant hyperglycemia and hyperlipidemia with the diabetogenic effect of STZ.[30,10] which are similar to human type 2 diabetes. Increased TG and TC are known markers of hyperlipidemia in HFD/STZ-induced diabetic mice.[31] Whereas levels of BUN and CREA, which are considered as significant markers of renal insufficiency. Urea is the major nitrogen containing metabolic product of protein metabolism; creatinine is endogenously produced and released into body fluids and its clearance measured as an indicator of renal function.[32] Treatment with hederagenin significantly decreased these parameters which could be due to decreased disturbances in protein and nucleic acid metabolism owing to better glycaemic control. However, it is still unclear which component is involved in this protection.

Hyperglycemia is a well-known cause for elevated free radical levels, followed by production of reactive oxygen species (ROS), which can lead to increased lipid peroxidation, alter antioxidant defense and further impair glucose metabolism in biological system.[33] Chronic treatment with hederagenin significantly improved the levels of endogenous antioxidant enzymes (GSH, CAT and SOD) and prevented membrane damage by decreasing lipid peroxidation compared to diabetic control. Histopathological study of kidney showed that both hederagenin and pioglitazone mesangial cell number was slightly higher in STZ (7.B). The degree of tubulointerstitial damage was modest. There were only few widened tubuli with incipient atrophy of the epithelial cells. In addition, slight focal interstitial fibrosis was observed. Intrarenal arterial vessel showed modest thickening of the walls.

The effects of streptozotocin on glucose homeostasis reflect the toxin induced abnormalities in β-cell function.[34] The therapeutic advantage of hederagenin was also reflected in the

pancreatic histology of mice supplemented with hederagenin where the damage induced on the islet and β-cells was minimal and preserved the islet architecture of the pancreas (Fig. 7.A).

Chronic supplementation of PM1 for a period of 28 days did not alter hematological parameters (RBC, WBC, platelet, hemoglobin, % hematocrit, MCV, MCH and MCHC) which were within the normal physiological ranges. There was no detectable hematological toxicity at therapeutic dose. The hederagenin have already been reported to be therapeutically useful in diabetic conditions and have been found to improve dyslipidemia in animals by addressing multiple targets associated with the pathology of DM.[15] Multi-targeted approaches, such as those discussed in the current paper, multiply the number of pharmacologically relevant targets by introducing a set of indirect, network dependent cascade with fewer side effects and toxicity. Hederagenin is beneficial in controlling diabetes, abnormalities in lipid profiles and oxidative stress by activation of enzymatic and non-enzymatic antioxidants in diabetic mice, and thus exemplify an approach with potential therapeutic value and balanced outcome.

In conclusion, the present study clearly demonstrates that the hederagenin from *Sapindus trifoliatus* is an effective antidiabetic agent with multiple therapeutic effects mediated by a combination of preventing the β-cell destruction, histological architecture of the pancreatic islets, improving glucose disposal by inhibiting DPP-IV and enhancing endogenous enzymatic antioxidant in liver.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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