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Original Article

PPAR-γ and GLUT-4 Mediated Insulin Sensitizing Action of *Paspalum scrobiculatum* Grains; An *in vivo* and *in silico* Study towards Validating it as a Long-Term Therapy for Type 2 Diabetes

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ABSTRACT

Purpose: Insulin Resistance (IR) usually results from a decline in levels of critical signalling proteins, their functional modifications, or both. Food and drinks rich in fat, energy and/or sugars increases the risk of obesity, promoting systemic IR and Type 2 Diabetes (T2D). Therapeutic approaches using natural compounds which promote and upregulate Glucose Transporter-4 (GLUT-4) and/or Peroxisome Proliferator Activated Receptors-γ (PPAR-γ), or increase adiponectin productions are advocated in treating T2D. The present study aimed to evaluate *in vivo* and *in silico* insulin sensitizing effect of *P. scrobiculatum* grains and to analyse the phytochemical composition using GC-MS technique. **Materials and Methods:** Sprague-Dawley rats were induced with IR by utilizing High Fructose and High Fat (HFHF) feed for 10 weeks. These rats were treated with fractions of *P. scrobiculatum* and pioglitazone for 6 weeks. Biochemical parameters, mRNA levels of PPAR-γ in adipose tissue and GLUT-4 in skeletal muscle were assessed. Further, GC-MS analysis of *P. scrobiculatum* followed by *in silico* screening towards GLUT-4 and PPAR-γ was performed. **Results:** Treatment with *P. scrobiculatum* significantly reduced Blood Glucose levels and normalized serum insulin, lipid and adiponectin levels. GLUT-4 and PPAR-γ mRNA levels appeared to be markedly elevated in the groups treated with Pioglitazone and *P. scrobiculatum*. GC-MS analysis of *P. scrobiculatum* revealed the presence of different constituents which were subjected to docking studies. This indicated 9,12-Octadecadienoic acid methyl ester and 9,17-Octadecadienal (Z)-showing good binding affinity towards PPAR-γ and GLUT-4. **Conclusion:** The present *in vivo* and *in silico* studies demonstrated insulin sensitizing action of *P. scrobiculatum* grains mediated by GLUT-4, PPAR-γ and adiponectin, thus concluding the multifaceted benefits in long-term treatment of T2D.

Keywords: Glucose transporter-4, High fructose High Fat feed, Insulin resistance, *Paspalum scrobiculatum*, PPAR-γ, Type 2 Diabetes.

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INTRODUCTION

Insulin Resistance (IR) is a clinical state where insulin exerts a declined biological response, usually results from a decline in levels of critical signalling proteins, their functional modifications, or both.[1] Chronic intake of food and beverages rich in fat, energy and/or sugars especially fructose increases the risk of obesity,[2] consequently promoting systemic IR and Type 2 Diabetes (T2D).[3] Therapeutic approaches using natural compounds which increase adiponectin production, promote Glucose Transporter-4 (GLUT-4) and/or Peroxisome proliferator activated receptors-γ (PPAR-γ) upregulation are highly advocated in the management of T2D.

Paspalum scrobiculatum Linn. grains (kodo millet) belonging to Poaceae family is considered as an ancient grain,[4] contains phenolics, proteins that are notable for their antioxidant activity

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and are also beneficial in the prevention and control of diabetes, cancer and cardiovascular diseases. *P. scrobiculatum* has been described in Ayurvedic texts and been used in folkloric and traditional medicine against diabetes mellitus, inflammatory diseases, corneal opacities, as a nutraceutical, as a tonic during typhoid fever and several other diseases.[5,6] Though there are some preliminary reports on the crude extracts of the plant as antidiabetic, no studies were performed at the molecular level to support these observations. In this context, we aimed to evaluate *in vivo* insulin sensitizing effect of *P. scrobiculatum* in high fructose and high fat feed induced insulin-resistance model and to study the phytochemical composition using GC-MS analysis, along with *in silico* screening towards PPAR-γ and GLUT-4.

MATERIALS AND METHODS

Drugs and chemicals

MMLV reverse transcriptase and primers have been obtained from M/S Sigma Chemical Co. The supplier of D-Fructose was M/S Loba Chemie Pvt. Ltd., located in Bangalore, India. The supplier of Pioglitazone was M/S Cipla Ltd., in Mumbai, India. M/S Crystal Chem. provided the rat insulin Elisa kit, whereas M/S Ray Biotech Inc. provided the rat adiponectin Elisa kit. M/S Thermo Fisher (USA) provided the TRIzol reagent and SYBR Green master mix. Kits from M/S Span Diagnostics Ltd., M/S Erba Diagnostics, Mumbai 400072, India, were used for every other biochemical estimates.

Plant material Collection, Extraction and Fractionation

P. scrobiculatum grains were procured, authenticated (specimen accession number-1259) and fractionated as reported in our previous study.[7] The two fractions obtained were labelled as HEPS (hexane:ethyl acetate (1:3) fraction of *P. scrobiculatum* grains) and EMPS (ethyl acetate:methanol (1:4) fraction of *P. scrobiculatum* grains).

In vivo assays

Animals

We purchased 150-180 g healthy male Sprague-Dawley rats from Sri Venkateswara Enterprises in Bangalore, India. The mice were kept in standard laboratory settings throughout the experiment, provided standard feed and RO-purified water *ad libitum*. The animals were used for the investigation following the adaption period. The current study methodology was reviewed and approved by Sri Venkateshwara College of Pharmacy's Institutional Animal Ethics Committee (IAEC) in Chittoor, India, under reference number 11-18/IAEC/CPCSEA/PO/SVCP/2017. Every research technique was carried out in compliance with the globally recognized guidelines for the use and care of laboratory animals.

Preparation of High Fructose and High Fat feed (HFHF)

High fructose solution was prepared by mixing 20% (w/v) fructose in drinking water. High fat feed was prepared by slight modification of the protocol used by Calvo-Ochoa *et al.*,[8] Munshi, *et al.*,[9] and Okoduwa *et al.*[10] Finely powdered premium high protein feed, which primarily consisted of 22% crude protein, 4% crude fat and 5% crude fibre, was mixed with 40% (w/w) Indian vanaspati (dalda) and given to the rats in the form of balls along with high fructose solution as drinking water *ad libitum*.

Induction of IR and Validation of the disease model for IR

Grouping of rats was done randomly into 2 groups, the Healthy Control (HC) group (n=6) which received standard pellet feed and RO purified water and the second, IR group of rats (n=42) were fed with freshly prepared high fat feed and high fructose solution *ad libitum* throughout the experimentation period *i.e.*, for 16 weeks.[11]

After ten weeks of High Fructose High Fat (HFHF) meal, IR was validated. In order to test the hyperglycaemic condition, all of the animals were fasted for 8 hr and their insulin and Fasting Blood Glucose (FBG) levels were measured. The Quantitative Insulin Sensitivity Check Index (QUICKI) and Homeostasis Model Assessment (HOMA-IR) were used to determine the degree of IR using the formulas below.[12]

HOMA-IR= [fasting glucose (mg/dL) X fasting insulin (μIU/mL)]/405

QUICKI=1/(log insulin (μIU/mL)+log glucose in mg/dL).

An increase in HOMA-IR,[13] with reduced QUICKI values (QUICKI ≤0.33) when compared to that of HC group, indicate the development of hyperglycemia and IR state.[9] Rats that did not meet the IR criteria were omitted from the study.

Experimental design

The rats meeting IR criteria were allocated randomly into six group sets (n=6) and were treated, along with the healthy control group daily as follows:

Group 1 served as Healthy Control (HC), received standard pellet feed and 1 mL 5% DMSO. Group 2 represented Disease Control (DC), received HFHF feed and 1 mL 5% DMSO. Group 3 was Standard Control (SC) group, fed with HFHF feed and administered Pioglitazone at a dose of 2 mg/Kg.[14] Group 4 was labelled as HEPS 100, fed with HFHF feed which received hexane:ethyl acetate fraction of *P. scrobiculatum* (HEPS) 100 mg/kg. Group 5 was HEPS 200, which received HFHF feed and HEPS at 200 mg/kg dose. Group 6 was named as EMPS 100, received HFHF feed and ethyl acetate:methanol fraction of *P. scrobiculatum* (EMPS) at 100 mg/kg dose. Group 7 named as

EMPS 200, provided with HFHF feed and 200 mg/kg of EMPS. The dosages of EMPS and HEPS (100 mg/kg and 200 mg/kg) were selected based on our previous study.[7] The test and standard compounds were dissolved in 5% DMSO 1 mL of the freshly prepared solutions were orally administered daily to the experimental animals as per the treatment schedule for 6 weeks. Weight of all the rats was monitored weekly.

Assessment of Biochemical Parameters and Tissue Collection

After 16 weeks of the experimentation, overnight, all of the rats were fasted, anesthetized by diethyl ether and killed. The blood samples were drawn carefully by cardiac puncture technique into collection tubes with and without anticoagulants for various biochemical investigations. Adipose tissue and skeletal muscle of hind limb were excised rapidly, washed using saline, freeze-clamped with liquid nitrogen and stored until required at -80ºC to determine protein expression of PPAR-γ in adipose tissue and GLUT-4 in skeletal muscle. FBG levels, serum insulin, adiponectin, Total Cholesterol (TC), Triglycerides (TG) and High-Density Lipoprotein cholesterol (HDL-C) were assessed. Furthermore, Low-Density Lipoprotein Cholesterol level (LDL-C), Very Low-Density Lipoprotein-Cholesterol level (VLDL-C), HOMA-IR and QUICKI were calculated.

Total RNA extraction and Real-Time PCR (RT-PCR)

The sampled tissues (each 100 mg) were extracted for total RNA with Trizol according to Villa-Rodríguez *et al.*[15] According to Suzuki *et al.*, the reverse transcriptase enzyme was used to synthesise complementary DNA (cDNA) for the RNA-isolated groups.[16] Using a particular set of primers, the cDNA was exposed to a polymerase chain reaction to amplify the genes. The sequence of oligonucleotide primers used for the assessment of gene expression levels (Supplementary Table S1). GAPDH was used as an internal control. Quantitative real-time Polymerase Chain Reaction (RT-qPCR) was executed by SYBR Green master mix in an RT-PCR instrument (Applied Biosystems 7500) as per manufacturer's protocol to achieve a final reaction volume of 20 µL. Each experiment was performed in triplicates and three samples were randomly selected from each group. The relative expression (fold change) of target gene was estimated by $2^{-\Delta\Delta Ct}$ method[17] and normalized according to the quantity of GAPDH (loading control) which has been validated as the housekeeping gene.[18]

GC-MS analysis

GC-MS was used for the identification of phytoconstituents of EMPS, following the standard procedure described in our previous report.[7]

In silico analysis

RCSB database was used to obtain the 3D structure of PPAR-γ (2PRG) and GLUT-4 was gathered from a developed homology model.[19] The resulting 3D structure was then processed using autodock tools.[20] The structure files (canonical smiles) of nineteen phytoconstituents including the standard drug Pioglitazone (Supplementary Table S2) were collected from Pubchem. Ten distinct runs with an exhaustiveness of 8 and a maximum of 250000 energy evaluations were used to perform each docking experiment. Docking simulations were performed using the Lamarckian Genetic Algorithm (LGA), the local search method developed by Solis and Wets and AMBER. During simulation studies, a fixed temperature of 298.15 K and a pressure of 1 atm were employed.[21] The results were visualised using Accelyrs discovery studio and both 2D and 3D images were generated. The binding affinities (in kcal/mol) and the count of potential hydrogen bonds, Vander Waals forces and other interactions were also determined.

Pharmacokinetic parameters by SwissADME

Using the online SwissADME server (http://www.swiss.adme.ch/), the pharmacokinetic characteristics of the bioactive substances were forecasted. Through the swissADME online database, the canonical SMILES saved from PubChem were utilised in this procedure. According to Daina *et al*,[22] the compounds were evaluated using Lipinski's Rule of five (RO5): (i) less hydrogen bond acceptor (10); (ii) less hydrogen bond donor (5); (iii) low molecular weight (500); (iv) high lipophilicity (Log P o/w, 5); and (v) TPSA refers to the topological polar surface area, measured in $Å^2$ (range of 0.0 to 450.0).

Statistical analysis

Each individual data element was shown as mean SD (n=6). The data in Microsoft Excel Spread Sheet for Windows (2010) were assessed using one-way Analysis of Variance (ANOVA), which was followed by an independent-sample t-test. Statistical significance was defined as p values below 0.05.

RESULTS

Validation of Insulin Resistance and effect of HEPS and EMPS in HFHF fed rats

After 10 weeks of HFHF feed, induction of IR was validated by assessing FBG and serum insulin levels. Rats fed with HFHF diet exhibited significantly higher levels of insulin and FBG than HC rats as shown in Table 1. The range of IR, represented by HOMA-IR was significantly elevated in HFHF fed rats than HC rats (9.0 vs. 1.3 respectively; Table 1). QUICKI Index was found to be decreased significantly in HFHF fed rats when compared to HC rats (0.28 vs 0.37 respectively). A spike in FBG, HOMA-IR[13] and insulin levels and reduced QUICKI values (QUICKI≤0.33) after the induction period, as compared to that of the HC group,

Table 1: Effect of HEPS and EMPS on FBG, serum insulin and insulin resistance markers in HFHF fed rats.

Groups	FBG (mg/dL)		Serum Insulin (µIU/mL)		HOMA-IR		QUICKI	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	after Treatment
HC	78.8±3.3	82.6±4.8	6.65±0.8	6.08±0.68	1.3	1.2	0.37	0.37
DC	240.8±9.5	268.3±7.1$^{\#}$	15.2±1.7	17.1±2.0$^{\#}$	9.0	11.64$^{\#}$	0.28	0.27$^{\#}$
SC	248.3±10.0	94.4±5.1**	14.7±1.5	7.78±0.8**	9.1	1.8**	0.28	0.35*
HEPS 100	244.0±10.4	133.3±5.9**	13.8±1.8	9.21±0.9**	8.4	3.0**	0.28	0.32*
HEPS 200	250.5±9.9	125.0±5.0**	15.1±2.0	8.9±0.8**	9.3	2.7**	0.28	0.33*
EMPS 100	256.2 ±8.2	114.5±4.9**	14.6±1.6	7.53±0.7**	9.4	2.1**	0.28	0.34*
EMPS 200	248.7±10.6	105.7±5.2	15.3±2.1	7.15±0.8**	9.4	1.9**	0.28	0.35*

Results are expressed in mean±S.D n=6; #p<0.0005 Diabetic Control (DC) compared to Healthy Control (HC); *p<0.005 treated group compared to diabetic control;**P<0.0005 treated group compared to diabetic control. [hexane:ethyl acetate (1:3) fraction of *P. scrobiculatum* grains (HEPS); ethyl acetate:methanol (1:4) fractions of *P. scrobiculatum* grains (EMPS).

indicate the development of hyperglycemia and IR state.[9] Thus, the present findings confirm and validate the induction of IR in HFHF fed rats, which are represented in Table 1 as before treatment.

Effect of HEPS and EMPS on FBG, serum insulin, HOMA-IR and QUICKI levels in HFHF fed rats

When compared to DC rats, HFHF-fed IR rats treated with HEPS, EMPS and pioglitazone for six weeks showed a significant decrease in FBG and serum insulin concentrations. Similarly, as compared to DC rats as indicated in Table 1 following treatment, the HOMA-IR values were dramatically reduced and QUICKI greatly improved upon treatment with HEPS, EMPS and pioglitazone. It was discovered that EMPS at 200 mg/kg was more successful in reducing IR brought on by HFHF diet.

Effect of HEPS and EMPS on body weight in HFHF fed rats

The HFHF feed during the induction period (10 weeks) increased body weight of all rats when compared to HC group as represented in Supplementary Table S3. The cumulative upsurge in food intake was correlated with the increase in body weight (observed, results not shown). In comparison to the HFHF-fed (DC) rats, the treatment groups' body weights were noticeably lower. In multiple comparisons, EMPS and HEPS at a dose of 100 mg/kg and 200 mg/kg doses exhibited a dose-dependent effect on body weights. The effect of EMPS and HEPS at 200 mg/kg was superior than pioglitazone (SC) treated rats. Furthermore, EMPS showed superior activity in lowering the body weight among all the tested groups.

Effect of HEPS and EMPS on Lipid parameters in HFHF fed rats

The serum TC, TG, LDL-C and VLDL-C values were elevated and HDL-C was declined markedly in DC rats compared to HC rats as represented in Table 2. Treatment with HEPS, EMPS and pioglitazone normalized the serum lipid levels in HFHF fed IR and hyperlipidemic rats. Correction of hyperlipidemia was the most significant outcome of HEPS and EMPS treatment, which was found to be comparable to pioglitazone (SC).

Effect of HEPS and EMPS on serum adiponectin levels in HFHF fed rats

The serum adiponectin level of the DC group was found to be significantly reduced relative to the HC group. After treatment with HEPS, EMPS and pioglitazone, serum adiponectin levels were significantly higher than those of the DC group as shown in Table 2.

Effect of HEPS and EMPS on mRNA expression level of GLUT-4 in the skeletal muscle of HFHF fed rats using RT-qPCR analysis

Relative mRNA expression (fold change) was calculated from the Ct-values by $2^{-\Delta\Delta Ct}$ method according to the quantity of reference gene, GAPDH. The Ct-value of DC was found to be high, whereas the treated and standard control group shown relatively low Ct-values. mRNA expression levels of GLUT-4 in the skeletal muscle of HFHF fed DC rats were found to be downregulated. In contrary, there was a 2.5-fold and 3-fold increase in the relative GLUT-4 mRNA expression in HEPS 200 and EMPS 200 treated rats respectively. Similarly, SC rats (pioglitazone 2 mg/kg) showed a 4.3-fold increase in the relative GLUT-4 mRNA expression as depicted in Figure 1.

Effect of HEPS and EMPS on mRNA expression level of PPAR-γ in adipose tissue of HFHF fed rats

DC rats fed with HFHF diet showed low PPAR-γ expression in the adipose tissue. Administration of EMPS, HEPS at 200 mg/kg showed 2.8-fold and 4.6-fold rise in the relative PPAR-γ mRNA expression levels respectively, which is comparable to the SC rats with 5.7-fold increase as represented in Figure 2.

Characterization of the phytochemical composition of EMPS by GC-MS analysis

Fractionation of methanol extract of *P. scrobiculatum* grains yielded two fractions viz., HEPS and EMPS. Since EMPS showed better outcome in the current *in vivo* study, it was further studied for the composition of phytoconstituents. GC-MS study of EMPS exposed the presence of n-Hexadecanoic acid (20.58%); 6-Octadecenoic acid (16.04%); Octadecanoic acid (2.54%) and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (4.71%) as major compounds as seen in Figure 3 along with other phytoconstituents as presented in Table 3. Other important bioactive compounds present in EMPS are N-carbobenzyloxy-cysteinylcysteine; 1,3-Dioxane 2-methyl-9,17-Octadecadienal, (Z)-9-Octadecenoic acid (Z)-methyl ester; Pentadecanoic acid, methyl ester. These compounds were reported for anti-oxidant, antitubercular, anti-inflammatory, hypocholesterolemic, anti-microbial, anti-cancer, anti-diabetic activities as depicted in Table 3.[23-25]

In silico Molecular Docking studies towards PPAR-γ and GLUT-4

Nineteen phytoconstituents of EMPS that are presented in Table 3 were studied using Pioglitazone and Neferine as standard drugs against PPAR-γ and GLUT-4 respectively. The study revealed that most of the compounds demonstrated binding to the active interaction site of PPAR-γ and GLUT-4. Seven phytoconstituents made the short list after the data were further examined by taking into account the docking scores and binding orientation of ligands towards PPAR-γ and GLUT-4.

The details of docking scores and the residues involved in the binding orientation of protein-ligand docked complexes towards PPAR-γ and GLUT-4 are presented in Table 4. Visual evaluation of the top docked compounds for 2D and 3D interactions with amino acid residues revealed the involvement of respective active

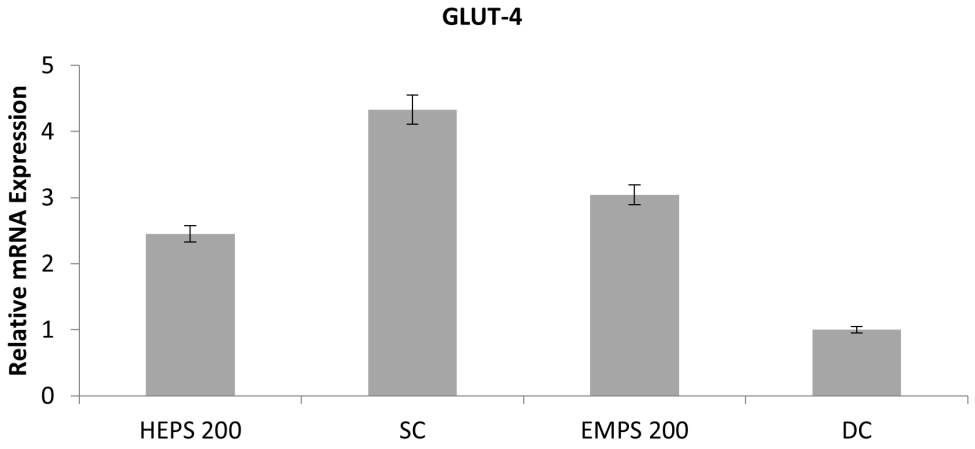

Figure 1: Effect of HEPS, EMPS and SC (Pioglitazone 2 mg/kg) treatments on the relative mRNA expression of GLUT-4 in skeletal muscle. Data is presented as mean±SD (*n*=3).

Table 2: Effect of HEPS and EMPS on adiponectin and lipid parameters in HFHF fed rats.

Groups	Adiponectin (µg/mL)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
HC	7.32±0.19	98.09±4.82	105.61±6.53	51.40±2.40	25.57±1.10	21.12±1.31
DC	2.91±0.16$^{\#}$	207.14±9.01$^{\#}$	292.53±10.78$^{\#}$	28.79±3.51$^{\#}$	119.3±3.34$^{\#}$	58.51±2.16$^{\#}$
SC	8.86±0.18**	129.36±5.28*	135.17±5.51**	44.85±1.91*	57.74**	27.03±1.10**
HEPS 100	4.31±0.2*	141.25±4.96*	170.29±6.85**	37.93±1.50*	69.26±2.09*	34.06±1.37*
HEPS 200	5.92±0.15**	136.70±5.74*	162.54±6.27**	40.36±1.97*	61.80±2.52*	32.51±1.25*
EMPS 100	5.70±0.17**	117.12±6.53*	147.80±6.01**	43.09±2.10*	23.6±2.82**	29.56±1.20*
EMPS 200	7.48±0.18**	104.54±4.71**	134.23±5.13**	45.65±2.66*	21.04±5.51**	26.85±1.03**

Results are expressed in mean±S.D *n*=6; #p<0.0005 Diabetic Control (DC) compared to Healthy Control (HC); *p<0.001 treated group compared to diabetic control; **p<0.0005 treated group compared to diabetic control.

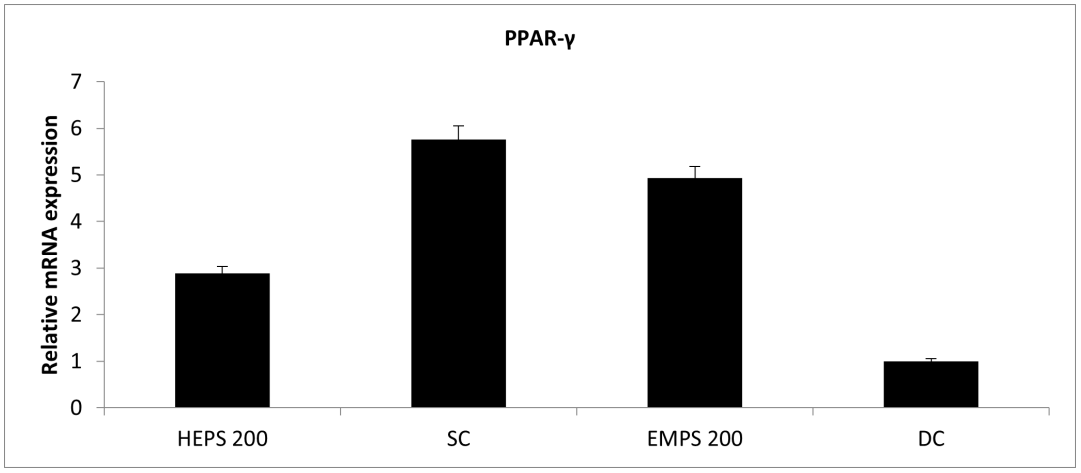

Figure 2: Effect of HEPS, EMPS and SC (Pioglitazone 2 mg/kg) treatments on the relative mRNA expression of PPAR-γ in the adipose tissue. Data is presented as mean±SD (*n*=3).

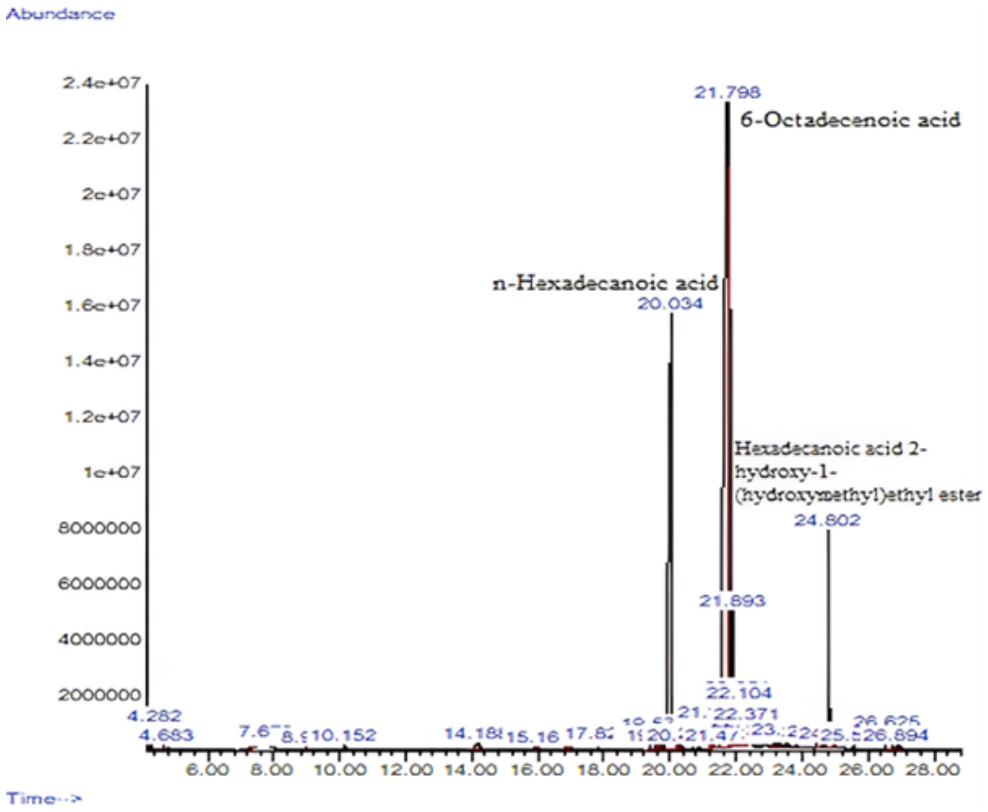

Figure 3: GC-MS chromatogram of EMPS.

binding sites of PPAR-γ (as shown in Figures 4 and 5) and GLUT-4 (as visualised in Figures 6 and 7). Of all the compounds screened towards PPAR-γ, 9,12-Octadecadienoic acid methyl ester and 9,17-Octadecadienal (Z) showed good docking scores of -6.6 and -6.4 kcal/mol respectively, as compared to that of the standard drug Pioglitazone, as represented in Figure 4a, with a docking score of -8.4 kcal/mol (Table 4). Similarly, as shown in Table 4, 9,12-Octadecadienoic acid methyl ester and 9,17-Octadecadienal (Z) showed good docking scores (-6.6, -6.6 kcal/mol respectively) against GLUT-4, which can be compared to Neferine as viewed in Figure 6a (-8.3 kcal/mol).

Pharmacokinetic properties

Lipinski's Rule of five (RO5), which includes molecular weight, rotatable bonds, hydrogen bond acceptor, hydrogen bond donor and Log P octanol-water partition coefficient, was used to assess the molecules' physicochemical characteristics. Swiss-ADME's results for intestinal absorption (low), permeability (-8 to -13), blood-brain barrier penetration, log P range (-100 to 3), lead-likeliness (1 to 2), bioavailability (0.11-0.55) and compound solubility were all below the permissible levels shown in Supplementary Table S4. Supplementary Table S5 display the outcomes of every other chemical that was examined.

Table 3: Phytoconstituents of EMPS and their reported biological activities.

RT (min)	%Area	CAS #	Compound Name	Reported Biological activities
4.282	0.23	189388-08-7	N-carbobenzyloxy-cysteinylcysteine	LT analogues
4.683	0.06	000507-09-5	Methanecarbothiolic acid	Antimicrobial
7.234	0.04	001874-62-0	3-Ethoxy-1,2-propanediol	-
7.476	0.10	000056-81-5	Glycerin	-
8.990	0.08	023899-73-2	4,5-Diamino-2-hydroxypyrimidine	Purine analogue
10.153	0.08	002117-34-2	Silane, triethylmethoxy-	-
14.187	0.54	1000303-59-8	4H-Pyrazole 3-tert-butylsulfanyl-4, 4-bistrifluoromethyl-	-
15.993	0.16	1000127-87-1	3-Deoxy-d-mannoic lactone	Anti-bacterial, Antioxidant
16.925	0.19	000626-68-6	1,3-Dioxane 2-methyl-	Hypolipidemic and hypoglycemic, PPAR alpha agonist
16.980	0.06	000542-90-5	Thiocyanic acid ethyl ester	-
17.820	0.09	000544-63-8	Tetradecanoic acid	Larvicidal, Anti-bacterial, antitumor activity
19.418	0.11	014202-13-2	3-Methyl-1-adamantaneacetic acid	Antiviral
19.714	0.19	1000351-74-3	Nonyl trifluoroacetate	-
20.034	20.58	000057-10-3	n-Hexadecanoic acid	Anti-inflammatory, hypocholesterolemic antispasmodic, anticancer and antiviral, hemolytic, 5-Alpha reductase inhibitor
20.262	0.12	056554-35-9	9,17-Octadecadienal (Z)-	Antimicrobial, Anti-inflammatory, antioxidant, MAO-A inhibitors
21.197	0.34	002462-85-3	9,12-Octadecadienoic acid methyl ester	-
21.255	0.41	000112-62-9	9-Octadecenoic acid (Z)- methyl ester	Anti-inflammatory, Antiandrogenic, cancer preventive, dermatitigenic, hypocholesterolemic, 5-alpha reductase inhibitor, Anemiagenic
21.476	0.02	007132-64-1	Pentadecanoic acid methyl ester	Diabetes and heart disease
21.799	16.04	1000336-66-8	6-Octadecenoic acid	-
21.895	2.54	000057-11-4	Octadecanoic acid	Antimicrobial
21.752	48.05	000060-33-3	9,12-Octadecadienoic acid (Z,Z)-	Antiarthritic, Anti-inflammatory, cancer preventive, hypocholesterolemic
23.517	0.15	000506-30-9	Eicosanoic acid	Antioxidant
24.436	0.08	001008-18-0	Naphthalene, 1,2,3,4-tetrahydro-1-meth oxy-	Antipyretic, Antiseizure, Anti-inflammatory
24.578	0.13	000934-10-1	3-Phenylbut-1-ene	-
24.803	4.71	023470-00-0	Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	Antioxidant, anti-inflammatory, anthelmintic
24.966	0.11	027554-26-3	1,2-Benzenedicarboxylic acid, diisooctyl ester	Antimicrobial, Antifungal
25.578	0.06	000629-50-5	Tridecane	-
26.626	0.98	085896-31-7	13-Tetradecenal	Anti-inflammatory, Radical scavenging
26.895	0.19	000621-61-4	Octadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	-

RT=Retention Time.

DISCUSSION

This study's primary objective was to determine whether improving insulin sensitivity in skeletal muscle and adipose tissues could mediate the anti-hyperglycemic effect of *P. scrobiculatum* grains. In humans, excessive consumption of food and drinks with high fructose and high fat reduces insulin sensitivity leading to the elevation of plasma triglycerides and lipids. Consequently, this contributes to obesity and accompanying metabolic abnormalities such as IR, consequently leading to T2D and hypertriglyceridemia.[2,3] The administration of a mixture of fructose and lipids was observed to be best experimental model to induce T2D.[2] Hence, in this study, we employed High Fructose and High Fat (HFHF) feed induced insulin resistance and hyperlipidemia as the preclinical model for screening of safe and effective insulin sensitizers against IR, T2D and hyperlipidemia.

The present results indicate that the HFHF fed rat model has effectively permitted the development of IR, T2D and allied long-term metabolic disturbances, which imitate the clinical parameters present in humans including increased body weight, hyperglycemia, hyperinsulinemia, hypertriglyceridemia and hypercholesterolemia. The fasting glucose and insulin levels reflect HOMA-IR and QUICKI index values which were used to quantify the IR and Insulin Sensitivity (IS) respectively. The induction of IR was further validated by the significant increased levels of HOMA-IR and reduced QUICKI index levels in comparison with Healthy Control (HC) rats. This was in agreement with previous reports which induced IR and hyperlipidemia using HFHF diet.[8,9]

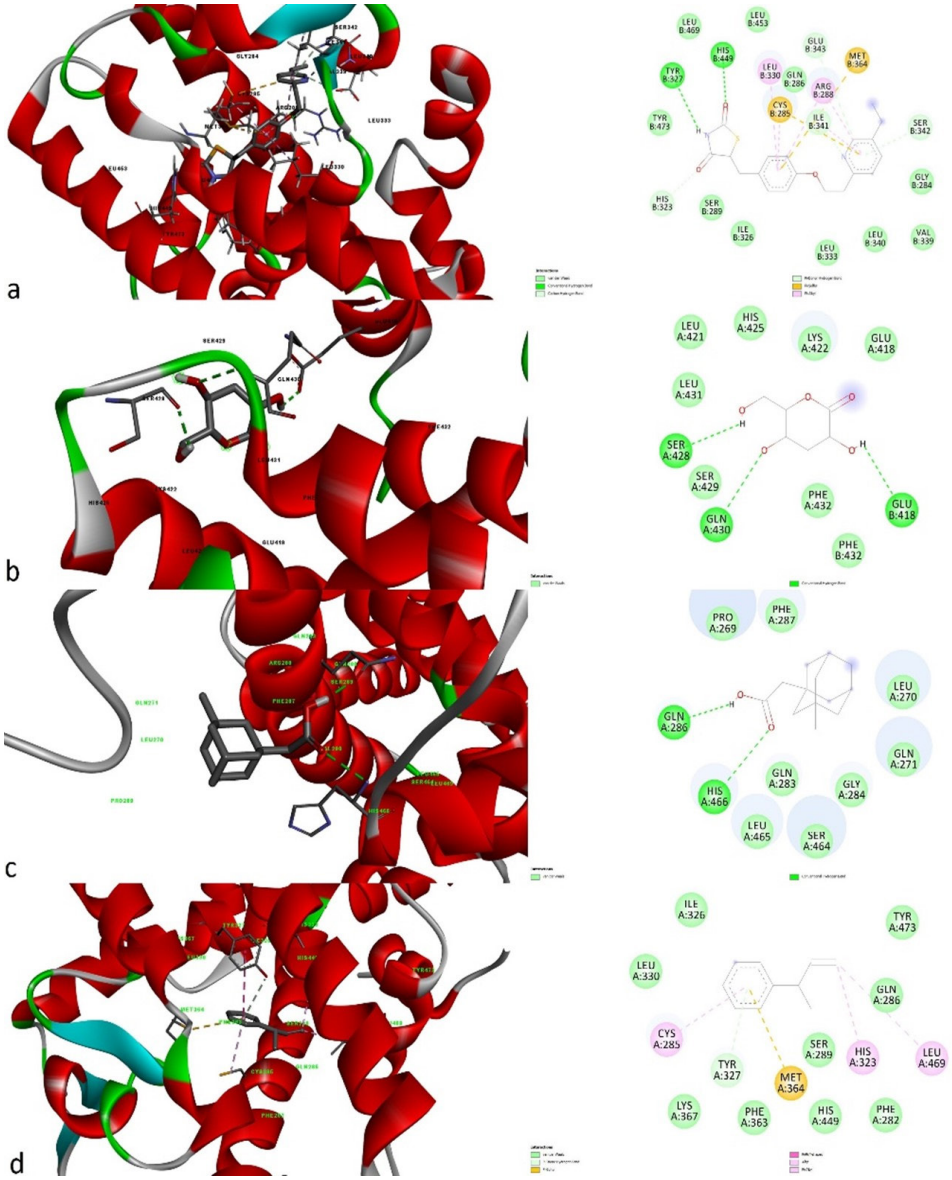

Figure 4: The docking complexes of PPAR-γ with (a) Pioglitazone (standard reference) (b) Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester (c) Octadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester (d) Methanecarbothiolic acid and the proposed molecules depicting the interactive site residues.

Table 4: Elucidation of docking scores and the amino acid residues involved in the binding orientation of protein-ligand docked complexes by docking studies against PPAR-γ and GLUT-4.

Sl. No.	Compound	Docking Score (kcal/mol)	H-bond residues	Van der Waals-interactions	Other interactions
			PPAR-γ		
1	PioglitazonePubChem ID: 4829	-8.4	TYR 327, HIS 449,	LEU 469, LEU 453, TYR 473, HIS 323, SER 289, ILE 326, LEU 333, LEU 340, VAL 339, GLY 284, SER 342ILE 341, GLN 286, GLU 343	LEU 330, ARG 288, CYS 285, MET 364
2	Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl esterPubChem ID:537294	-5.9	GLN 286, HIS 466.	PRO 269, PHE 287, GLN 283, LEU 465, SER 464, GLY 284, GLN 271, LEU 270	-
3	Octadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl esterPubChem ID: 129853056	-6.3	GLN 286, HIS 466	PRO 269, PHE 287, LEU 270, GLN 271, GLY 284, SER 464, GLN 283, LEU 465	-
4	Methanecarbothiolic acidPubChem ID: 10484	-5.7	-	ILE 326, LEU 330, LYS 367, PHE 363, SER 289, HIS 449, TYR 473, GLN 286, PHE 282	CYS 285, TYR 327, MET 364, HIS 323, LEU 469
5	4,5-Diamino-2-hyd roxypyrimidinePubChem ID: 248637	-5.7	GLN 286	PHE 282, MET 463, SER 464, GLN 470, LYS 474, GLN 454	ILE 456, LEU 465, LEU 453, LYS 457, VAL 450, TYR 473
6	3-Deoxy-d-mannoic lactonePubChem ID: 541561	-6.0	ASP 462, MET 463.	THR 461, GLU 460, SER 464, PHE 282, ILE 456, GLN 286, GLN 454, LYS 474, GLN 470	LEU 465, LEU 453, LYS 457, TYR 473.
7	9,17-Octadecadienal (Z)-PubChem ID: 5365667	-6.4	CYS 285	ILE 341, LEU 340, GLU 343, SER 342, LEU 333, VAL 339, ILE 326	ARG 288, LEU 330
8	9,12-Octadecadienoic acid methyl esterPubChem ID: 8203	-6.6	CYS 285, SER 289, TYR 327	ILE 281, LEU 353, MET 364, LEU 330, GLY 284, ARG 288, PHE 363, GLN 286, ILE 326, HIS 323, HIS 449, TYR 473	VAL 339, MET 348, ILE 341, PHE 282.
			GLUT-4		
9	Nefirine	-8.3	GLU 396, GLN 298, SER 153	PHE 395, GLY 400, GLY 154, MET 420, ASN 176, ALA 421, THR 337, GLN 177, PHE 38, TRP 428, SER 96, GLY 424	ILE 99, ILE 180, TRP 404
10	Octadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl esterPubChem ID: 129853056	-6.9	PRO 417	ALA 421, SER 96, GLY 424, PHE 425, SER 153, GLY 154	TRP 404, MET 420, ILE 99
11	Methanecarbothiolic acid PubChem ID: 10484	-5.7	-	GLN 299, ASN 431, ASN 304, ASN 333, ILE 184, ILE 180, GLU 396	ILE 42, ILE 303, PHE 395, PHE 307
12	3-Deoxy-d-mannoic lactone PubChem ID: 541561	-5.8	-	ASN 431, GLN 299, ASN 427, GLY 424, MET 420, ILE 180, ILE 303, ASN 333, SER 153, SER 96, ILE 99, ALA 421	ILE 42, TRP 428, PHE 38, ILE 184, TRP 404, PHE 307
13	9,17-Octadecadienal (Z)-PubChem ID: 5365667	-6.6	-	PRO 157, MET 420, GLY 154, SER 153, PHE 38, GLY 424, ASN 427	TRP 428, TRP 404
14	9,12-Octadecadienoic acid methyl ester PubChem ID: 8203	-6.6	ASN 427, GLN 299	SER 153, TRP 428, ASN 431, ASN 304, PHE 307, TRP 404, GLN 298, GLU 396, ILE 303, ILE 180, GLN 177, PRO 402, ILE 184	PHE 38, PHE 395, ILE 42

15	Pentadecanoic acid methyl ester PubChem ID: 23518	-6.1	TRP 428, SER 153, SER 96	ASN 276, GLN 177, PRO 401, MET 420, PHE 38, GLY 424	PHE 405, TRP 404, ASN 427
16	Octadecanoic acid PubChem ID: 5281	-5.9	ASN 431	ASN 427, GLN 299, GLN 298, SER 153, ASN 176, ASN 333, GLU 396, ASN 304	ILE 180, TRP 404, PHE 38, ILE 42, PHE 395, PHE 307, ILE 184, ILE 303

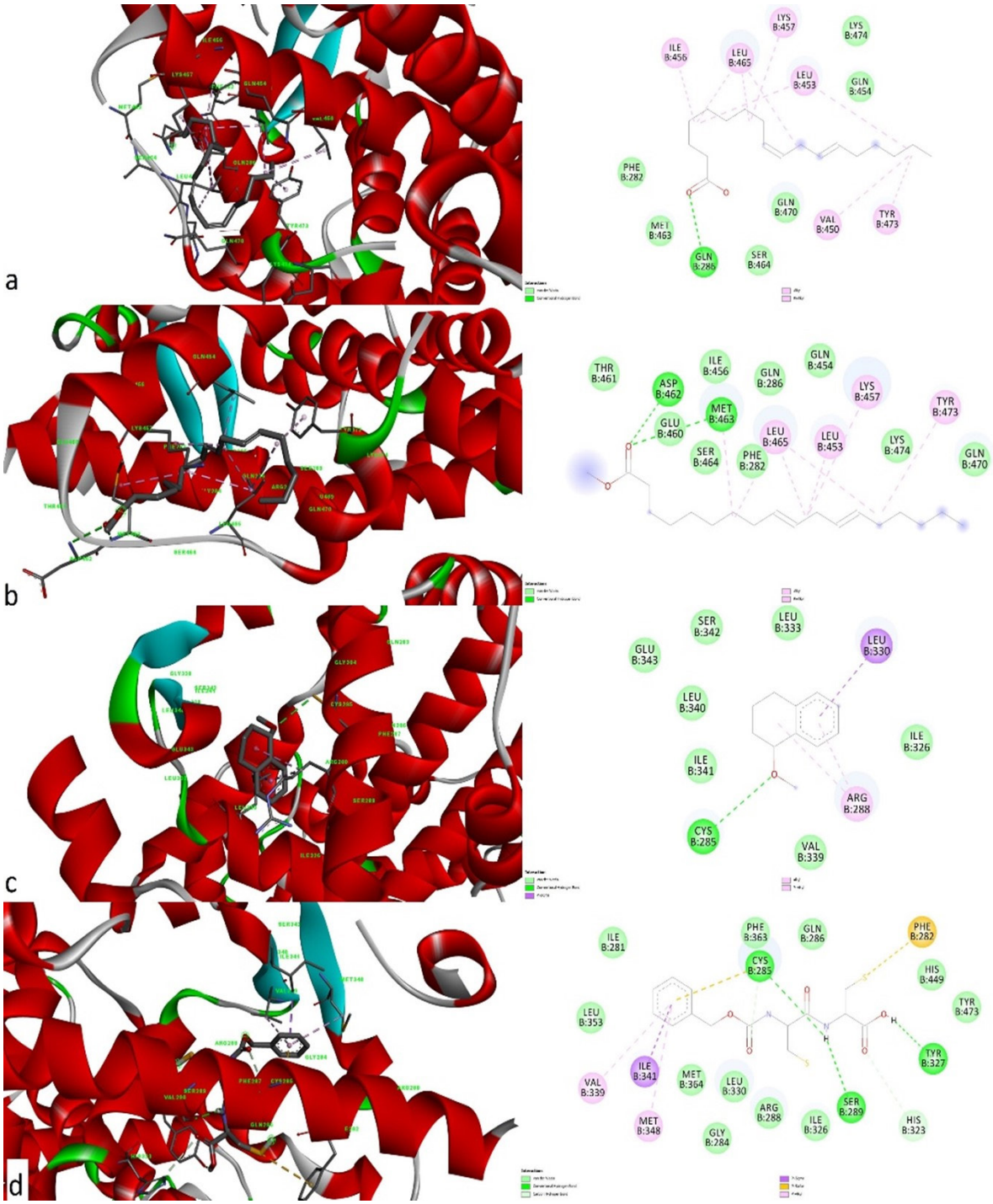

Figure 5: The docking complexes of PPAR-γ with (a) 4,5-Diamino-2-hydroxypyrimidine (b) 3-Deoxy-d-mannoic lactone (c) 9,17-Octadecadienal (Z)-(d) 9,12-Octadecadienoic acid methyl ester and the proposed molecules depicting the interactive site residues.

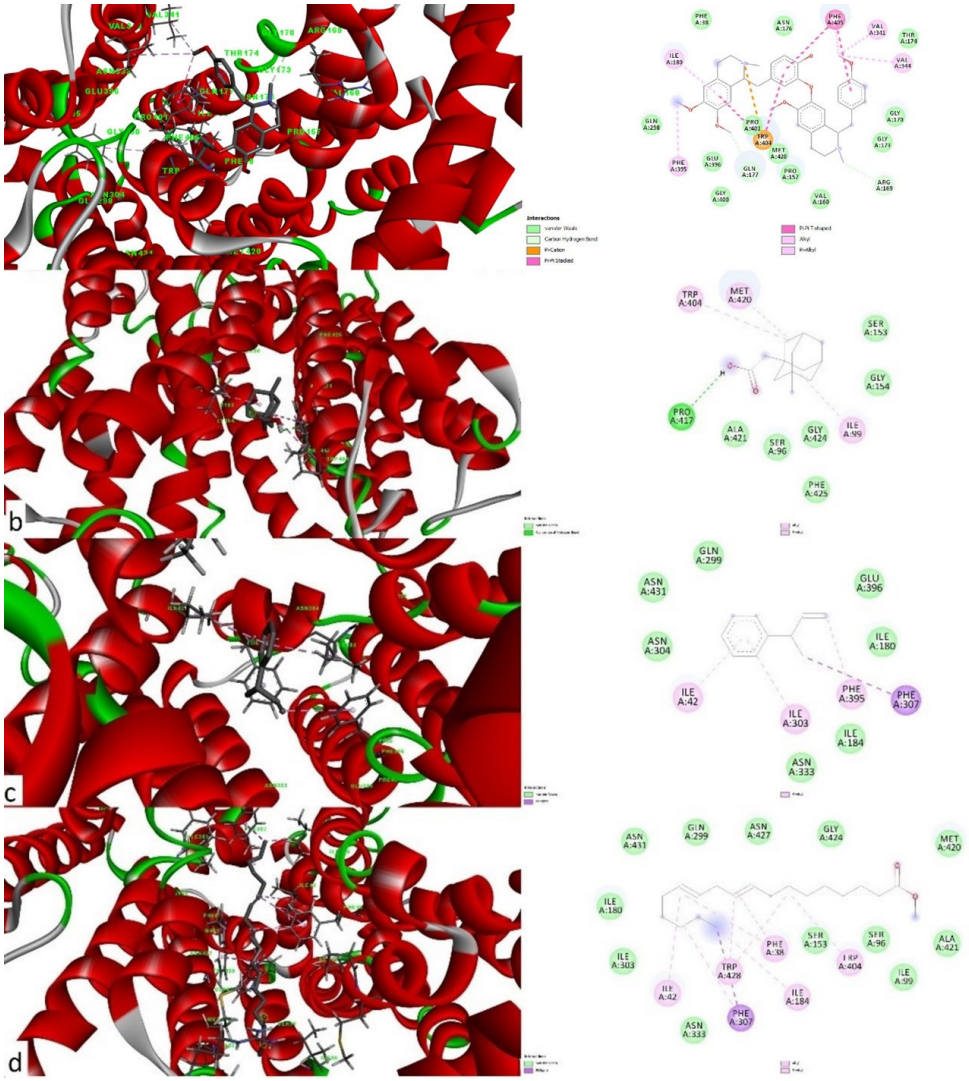

Figure 6: The GLUT-4 docked complexes with (a) Niferine (standard reference) (b) Octadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester (c) Methanecarbothiolic acid (d) 3-Deoxy-d-mannoic lactone and the proposed molecules with the representation of active pocket.

The present study used thiazolidinedione class of drugs namely pioglitazone 2 mg/kg as standard reference, which is a strong ligand for PPAR-γ that exert insulin-sensitizing effects by altering the gene expression.

In the present study, administration of HEPS and EMPS at both doses for 6 weeks reversed the features of IR that are seen in HFHF fed DC rats. The current results are in accordance with the previous study that showed insulin sensitizing effect in HFHF fed rats.[13] Additionally, HFHF diet resulted in hyperlipidaemia, which may have been brought on by an excess of free fatty acids that accumulated in the liver and were eventually converted to Triglycerides (TG).[26] One important stage in the underlying hypertriglyceridemia of IR is the fat cell's impaired capacity to retain excess TG.[27] From the present results, HFHF feed induced the anticipated rise in body weight significantly. Nevertheless, HEPS and EMPS administration not only prevented the rise in body weight but also decreased it to near normal levels. HEPS, EMPS and pioglitazone normalized serum lipid levels suggesting anti-obesity action of these fractions dose-dependently.

Obesity also modifies the secretion of adipocytokines, prominently adiponectin, which acts by increasing insulin sensitivity and drops FBG in diabetic subjects, by improving insulin-regulated inhibition of gluconeogenesis.[28] In the current study, the rise in the adiponectin level by HEPS and EMPS could be correlated to their protective effect in alleviating IR.[29]

The underlying biological pathways were better understood by looking at the expression of the PPAR-γ and GLUT-4 genes in skeletal muscle and adipose tissue, respectively. By reducing lipotoxicity, controlling the expression of adiponectin and leptin, which affect insulin sensitivity in the liver and skeletal muscle and enhancing the activity and survival of pancreatic β cells, PPAR-γ improves insulin sensitivity throughout the body.[30] Activation of PPAR-γ stimulates energy storage in white adipose tissue, thereby sensitizing liver and peripheral tissues to insulin. GLUT-4 is a

major glucose transporter involved in insulin mediated glucose uptake in skeletal muscle and adipocytes. GLUT-4 upregulation can enhance the translocation into the cell membrane thereby increasing the glucose uptake thus regulates glucose homeostasis in the body. Downregulation of GLUT-4 expression is a prominent feature of IR states, leading to diminished glucose uptake into the target tissues.[31,32]

As there was a clear dose-dependent action evident from the present biochemical results, high dose of the fractions was selected for assessing the effect on mRNA expression levels. In the present study, upregulation of PPAR-γ mRNA expression in HEPS and EMPS treated groups could be linked to the decrease in serum lipid parameters and increased adiponectin levels thus suggesting the insulin sensitizing effect. The present upregulation of GLUT-4 expression could be correlated with the marked decrease of blood glucose levels, confirming that HEPS and EMPS could regulate glucose homeostasis and insulin action in the body. The present findings thus support the involvement of PPAR-γ and GLUT-4 in the insulinomimetic and/or insulin sensitizing effect of HEPS and EMPS. Moreover, HEPS and EMPS might enhance glucose tolerance and insulin-dependent glucose uptake, consequently preventing IR,[33,34] which could be the possible mechanism for the antidiabetic and antihyperlipidemic activities.[35]

Since EMPS has shown better results, GC-MS analysis was performed only on EMPS. The results, for the first time, revealed different bioactive phytoconstituents like n-Hexadecanoic acid; 6-Octadecenoic acid; Octadecanoic acid and Hexadecanoic

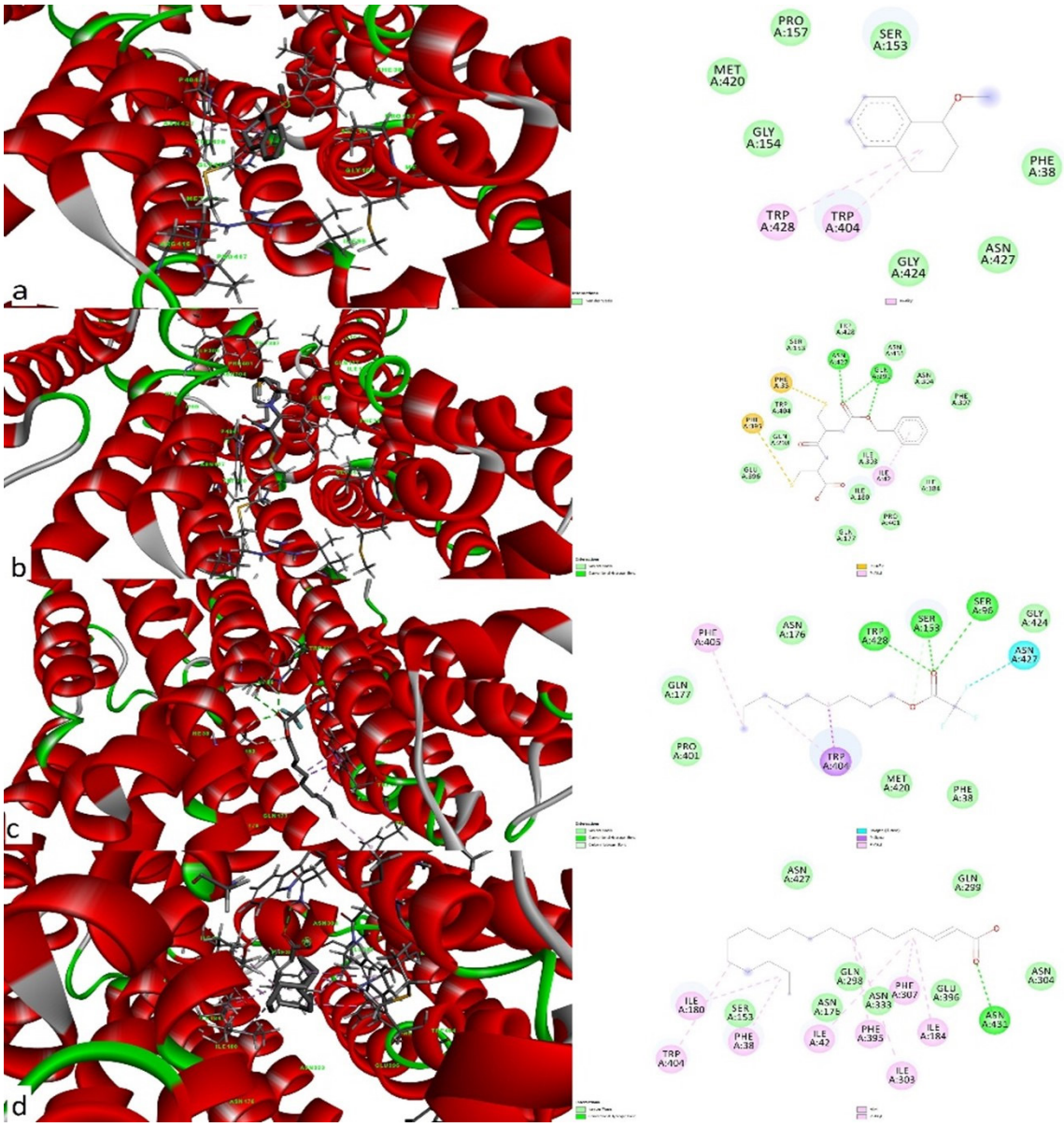

Figure 7: The GLUT-4 docked complexes with (a) 9,17-Octadecadienal (Z)-(b) 9,12-Octadecadienoic acid methyl ester (c) Pentadecanoic acid methyl ester (d) Octadecanoic acid and the proposed molecules with the representation of active pocket.

acid,2-hydroxy-1-(hydroxymethyl) ethyl ester as prominent compounds.

Database search revealed that PPAR-γ has active ligand binding sites involving amino acid residues ILE 281, 282, GLY 284, CYS 285, GLN 286, 288, 289, 323, 326, 327, SER 342, MET 348, LEU 330, PHE 363, MET 364, VAL 339, LEU 340, LEU 469, TYR 473.[36] On the other hand, 3D structure of GLUT-4 was not available in RCSB database so a homology model generated by Wei *et al.*, was considered and utilized for the present study.[19] According to previous reports, amino acid residues PHE 38, GLY 170, GLY 173, ASN 176, GLN 177, GLN 298, ASN 304, ASN 333, VAL 341, PHE 395, GLU 396, TRP 404, PHE 405, ASN 431 were observed as key binding sites for GLUT-4.[37]

Along with docking score, bond interactions between ligand and amino acid residues of receptor are a crucial for optimum pharmacological activity. The active sites of receptors, which have been found to be the binding sites for agonist chemicals' effects on the corresponding receptors, have demonstrated a high affinity for the natural compounds.[38] The present docking results showed that 7 phytoconstituents each have shown good docking score towards the active binding sites of PPAR-γ and GLUT-4, indicating strong binding interaction. However, two compounds namely 9,12-Octadecadienoic acid methyl ester and 9,17-Octadecadienal (Z)- were found to show good binding affinities towards both PPAR-γ and GLUT-4 when compared with other screened molecules, revealing their importance in targeting the functions of PPAR-γ and GLUT-4 in enhancing insulin sensitivity and reducing lipotoxicity. These results indicate that the proposed compounds are likely to produce agonistic effects and thus can be used for further wet lab techniques.

The present study revealed that the screened molecules obeyed RO5, indicating good oral bioavailability and drug likeliness, thus these molecules can be considered as potential drug candidates with receptor-based optimization.

Further research including purification and isolation of the bioactive compounds from the fractions would be worthwhile in order to establish their real potential as phytotherapeutic agents.

CONCLUSION

The current study established the mode of action of *P. scrobiculatum* grains in T2D and hyperlipidemia in rats through *in vivo* and *in silico* methods. The present *in vivo* study revealed the insulin sensitizing action of *P. scrobiculatum* grains through increasing adiponectin levels, upregulation of GLUT-4 and PPAR-γ mRNA expression, thus efficiently improving glucose homeostasis in HFHF fed insulin resistant and hyperlipidemic rats. Furthermore, GC-MS study exposed the presence of many pharmacologically active phytoconstituents, which were identified for the first time in the fractions of *P. scrobiculatum* grains. In addition, *in silico* docking analysis predicted the possible agonistic role of the phytoconstituents on PPAR-γ and GLUT-4 like Pioglitazone and Neferine respectively. These results conclude the multifaceted benefits of the plant in preventing the progression of hyperlipidemia and IR to T2D and associated complications. Hence *P. scrobiculatum* grains can be included as part of dietary management and can be effectively used in the treatment and management of Insulin resistance, diabetes and hyperlipidemia thus justifying the traditional use.

ACKNOWLEDGEMENT

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ABBREVIATIONS

EMPS: Ethyl acetate:methanol (1:4) fractions of *P. scrobiculatum* grains; **FBG:** Fasting Blood Glucose; **GLUT-4:** Glucose Transporter 4; **HEPS:** Hexane:ethyl acetate (1:3) fraction of *P. scrobiculatum* grains; **HFHF:** High fructose solution and high fat feed; **HOMA-IR:** Homeostatic Model Assessment-Insulin Resistance; **IR:** Insulin Resistance; **PPAR-γ:** Peroxisome proliferator-activated receptor-γ; **QUICKI:** Quantitative insulin sensitivity check index; **RT-PCR:** Real time Polymerase chain reaction; **T2D:** Type 2 Diabetes mellitus.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

The Institutional Animal Ethics Committee (IAEC) of Sri Venkateshwara College of Pharmacy, Chittoor, India, examined and approved the current study protocol with reference number 11-18/IAEC/CPCSEA/PO/SVCP/2017.

SUMMARY

Following *P. scrobiculatum* treatment, serum insulin, cholesterol and adiponectin levels returned to normal and blood glucose levels were dramatically decreased. The groups treated with pioglitazone and *P. scrobiculatum* exhibited a considerable upregulation of GLUT-4 and PPAR-γ mRNA levels. GC-MS analysis and further docking studies revealed that 9,12-Octadecadienoic acid methyl ester and 9,17-Octadecadienal (Z)-have good binding affinity towards PPAR-γ and GLUT-4. Thus, we summarize, *P. scrobiculatum* has insulin sensitizing action with multifaceted benefits in long-term treatment of T2D.

REFERENCES

1. Awazawa M, Ueki K, Inabe K, Yamauchi T, Kubota N, Kaneko K. *et al.* Adiponectin enhances insulin sensitivity by increasing hepatic IRS-2 expression via a macrophage-derived IL-6-dependent pathway. Cell Metab. 2011; 13: 401-12. doi:10.1016/j.cmet.2011.02.010.
2. Lozano I, Van der Werf R, Bietiger W, Seyfritz E, Pinget M, Dal S. *et al.* High-fructose and high-fat diet-induced disorders in rats: impact on diabetes risk, hepatic and vascular complications. Nutr Metab (Lond). 2016; 13: 15. doi:10.1186/s12986-016-0074-1.
3. Hammarstedt A, Graham TE, Kahn BB. Adipose tissue dysregulation and reduced insulin sensitivity in non-obese individuals with enlarged abdominal adipose cells. Diabetol Metab Syndr. 2012; 4: 1-9. doi:10.1186/1758-5996-4-42.
4. Deshpande SS, Mohapatra D, Tripathi MK, Sadvatha RH. Kodo millet-nutritional value and utilization in Indian foods. J Grain Process Storage. 2015. Available from: www.jakraya.com/journal/jgps.
5. Ramasamy S, Sakthivel K, Kumar A, Ratnavathi C, Karunagaran D, Chandra T. *Paspalum scrobiculatum* polyphenol rich extracts inhibit human cervical cancer cell proliferation and induce apoptosis *in vitro*. FASEB J. 2017; 31: 162. doi:10.1096/fasebj.31.1_supplement.lb162.
6. Jain S, Bhatia G, Barik R, Kumar P, Jain A, Dixit VK. Antidiabetic activity of *Paspalum scrobiculatum* Linn. in alloxan-induced diabetic rats. J Ethnopharmacol. 2010; 127: 325-8. doi:10.1016/j.jep.2009.10.038.
7. Reddy GJ, Reddy KB, Reddy GV. Antidiabetic and antidyslipidemic activities of hexane/ethyl acetate/methanol fractions of *Paspalum scrobiculatum* Linn. grains in high-fat diet and streptozotocin-induced diabetic rats. Phcog Res. 2020; 12: 230-5.
8. Calvo-Ochoa E, Hernández-Ortega K, Ferrera P, Morimoto S, Arias C. Short-term high-fat-and-fructose feeding produces insulin signaling alterations accompanied by neurite and synaptic reduction and astroglial activation in the rat hippocampus. J Cereb Blood Flow Metab. 2014; 34: 1001-8. doi:10.1038/jcbfm.2014.48.
9. Munshi RP, Joshi SG, Rane BN. Development of an experimental diet model in rats to study hyperlipidemia and insulin resistance, markers for coronary heart disease. Indian J Pharmacol. 2014; 46: 270-6. doi:10.4103/0253-7613.132156.
10. Okoduwa SI, Umar IA, James DB, Inuwa HM. Appropriate insulin level in selecting fortified diet-fed, streptozotocin-treated rat model of type 2 diabetes for anti-diabetic studies. PLoS One. 2017; 12(1): e0170971. doi:10.1371/journal.pone.0170971.
11. Zayed EA, AinShoka AA, El Shazly KA, Abd El Latif HA. Improvement of insulin resistance via increase of GLUT4 and PPARγ in metabolic syndrome-induced rats treated with omega-3 fatty acid or l-carnitine. J Biochem Mol Toxicol. 2018; 32: e22218. doi:10.1002/jbt.22218.
12. Ezzat SM, El Bishbishy MH, Aborehab NM, Motaal AA, Hasheesh A, Rashad H. *et al.* Upregulation of MC4R and PPAR-α expression mediates the anti-obesity activity of Moringa oleifera Lam. in high-fat diet-induced obesity in rats. J Ethnopharmacol. 2020; 251: 112541. doi:10.1016/j.jep.2020.112541.
13. Pyo YH, Lee KW. Preventive effect of Monascus-fermented products enriched with ubiquinones on type 2 diabetic rats induced by a high-fructose plus high-fat diet. J Med Food. 2014; 17: 826-9. doi:10.1089/jmf.2013.3001.
14. Tayade PM, Jagtap SA, Borde S, Chandrasekar N, Joshi A. Effect of Psoralea corylifolia on dexamethasone-induced insulin resistance in mice. J King Saud Univ Sci. 2012; 24: 251-5. doi:10.1016/j.jksus.2011.03.007.
15. Villa-Rodríguez E, Ibarra-Gámez C, de Los Santos-Villalobos S. Extraction of high-quality RNA from *Bacillus subtilis* with a lysozyme pre-treatment followed by the Trizol method. J Microbiol Methods. 2018; 147: 14-16. doi:10.1016/j.mimet.2018.02.011.
16. Suzuki T, Ikeda H, Mase M. Restricted viral cDNA synthesis in cell lines that fail to support productive infection by bovine leukemia virus. Arch Virol. 2018; 163: 2415-22. doi:10.1007/s00705-018-3887-6.
17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25: 402-8. doi:10.1006/meth.2001.1262.
18. Bahrami G, Sajadimajd S, Mohammadi B, Hatami R, Miraghaee S, Keshavarzi S. *et al.* Anti-diabetic effect of a novel oligosaccharide isolated from Rosa canina via modulation of DNA methylation in Streptozotocin-diabetic rats. Daru. 2020; 28: 581-90. doi:10.1007/s40199-020-00363-8.
19. Wei C, Bajpai R, Sharma H, Matulis SM, Jain AD, Kalyanaraman B. *et al.* Development of GLUT4-selective antagonists for multiple myeloma therapy. Eur J Med Chem. 2017; 139: 573-86. doi:10.1016/j.ejmech.2017.08.029.
20. Bikadi Z, Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminformatics. 2009; 1: 15. doi:10.1186/1758-2946-1-15.
21. Kanipakam H, Sharma K, Thinlas T, Mohammad G, Pasha MAQ. Structural and functional alterations of nitric oxide synthase 3 due to missense variants associate with high-altitude pulmonary edema through dynamic study. J Biomol Struct Dyn. 2021; 39: 294-309. doi:10.1080/07391102.2019.1711190.
22. Daina N, Sivakumar A, Polak JW. Modelling electric vehicles use: a survey on the methods. Renew Sust Energ Rev. 2017; 68: 447-60. doi:10.1016/j.rser.2016.10.005.
23. Sharma A, Rai PK, Prasad S. GC-MS detection and determination of major volatile compounds in *Brassica juncea* L. leaves and seeds. Microchem J. 2018; 138: 488-93. doi:10.1016/j.microc.2018.04.013.
24. Singh R, Chaturvedi P. Phytochemical characterization of rhizome, fruit, leaf and callus of *Rheum emodi* Wall. using GC-MS. Pharmacogn J. 2019; 11(3): 12-19. doi:10.5530/pj.2019.3.4.
25. Abubakar MN, Majinda RR. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). Medicines. 2016; 3: 3. doi:10.3390/medicines3010003.
26. Silva Figueiredo P, Carla Inada A, Marcelino G, Maiara Lopes Cardozo C, de Cássia Freitas K, de Cássia Avellaneda Guimarães R, *et al.* Fatty acids consumption: the role metabolic aspects involved in obesity and its associated disorders. Nutrients. 2017; 9: 1158. doi:10.3390/nu9101158.
27. Roberts CK, Hevener AL, Barnard RJ. Metabolic syndrome and insulin resistance: underlying causes and modification by exercise training. Compr Physiol. 2013; 3: 1-58. doi:10.1002/cphy.c110062.
28. Combs TP, Berg AH, Obici S, Scherer PE, Rossetti L. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J Clin Invest. 2001; 108: 1875-81. doi:10.1172/JCI14120.
29. Neschen S, Morino K, Rossbacher JC, Pongratz RL, Cline G, Sono S. *et al.* Fish oil regulates adiponectin secretion by a peroxisome proliferator-activated receptor-gamma-dependent mechanism in mice. Diabetes. 2006; 55: 924-8. doi:10.2337/diabetes.55.04.06.db05-0985.
30. Dubois V, Eeckhoute J, Lefebvre P, Staels B. Distinct but complementary contributions of PPAR isotypes to energy homeostasis. J Clin Invest. 2017; 127: 1202-14. doi:10.1172/JCI88894.
31. Wu Z, Xie Y, Morrison RF, Bucher NL, Farmer SR. PPARgamma induces the insulin-dependent glucose transporter GLUT4 in the absence of C/EBPalpha during the conversion of 3T3 fibroblasts into adipocytes. J Clin Invest. 1998; 101: 22-32. doi:10.1172/JCI1244.
32. Berger J, Moller DE. The mechanisms of action of PPARs. Annu Rev Med. 2002; 53: 409-35. doi:10.1146/annurev.med.53.082901.104018.
33. Kim S, Jwa H, Yanagawa Y, Park T. Extract from Dioscorea batatas ameliorates insulin resistance in mice fed a high-fat diet. J Med Food. 2012; 15: 527-34. doi:10.1089/jmf.2011.2008.
34. Li Y, Goto T, Yamakuni K, Takahashi H, Takahashi N, Jheng HF. *et al.* 4-Hydroxyderricin, as a PPARγ agonist, promotes adipogenesis, adiponectin secretion and glucose uptake in 3T3-L1 cells. Lipids. 2016; 51: 787-95. doi:10.1007/s11745-016-4154-9.
35. Reifel-Miller A, Otto K, Hawkins E, Barr R, Bensch WR, Bull C. *et al.* A peroxisome proliferator-activated receptor α/γ dual agonist with a unique *in vitro* profile and potent glucose and lipid effects in rodent models of type 2 diabetes and dyslipidemia. Mol Endocrinol. 2005; 19: 1593-605. doi:10.1210/me.2005-0015.
36. Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R. *et al.* Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ. Nature. 1998; 395: 137-43. doi:10.1038/25931.
37. Dound YA, Chaudhary S, Chaudhary SS, Rawat S, Alqarni MH, Ahmad MM. *et al.* Mechanistic understanding of Ptyrone™: A plant-based natural anti-diabetic product. J King Saud Univ Sci. 2021; 33: 101454. doi:10.1016/j.jksus.2020.101454.
38. Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. *In silico* and *in vivo* analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. Toxicol Mech Methods. 2019; 29: 276-90. doi:10.1080/15376516.2018.1545815.

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Supplementary Table S1: Primers used in this study for real time PCR.

Batch No.	Oligonucleotide Name	Primer Sequence (5'-3')
BA01033644	GLUT 4	F: CTGGCGCTTTCACTGAACTR: CGAGGCCAAGGCTAGATTTTG
BA01033646	PPAR-γ	F: CATGCTTGTGAAGGATGCAAGR:TTCTGAAA CCGACAGTACTGACAT
BA01033648	GAPDH	F: TGGAGTCTACTGGCGTCTTR: TGTCATATTTCTCGTGGTTCA

Supplementary Table S2: Effect of HEPS and EMPS on body weight in HFHF fed rats.

Groups	Baseline weight (day 1) (g)	Initial Weight before treatment (After 10 weeks) (g)	Final Weight after treatment (After 16 weeks) (g)	Δ Weight Change (g)
HC	188±6.5	211±5.3	220±5.6	9
DC	186±5.8	250±10.7	279±11.4	29
SC	191±7.1	254±7.2	266±4.9	12
HEPS 100	183±5.6	246±7.9	249±6.2	3
HEPS 200	185±6.3	251±8.5	244±6.5	-7
EMPS 100	180±7.5	242±9.1	236±6.6	-6
EMPS 200	190±5.9	255±7.7	236±5.2	-19

Results are expressed in mean±S.D *n*=6.

Supplementary Table S3: Canonical smiles of the molecules

Ligand	Canonical smiles	Dock score PPAR Gamma (kcal/mol)	Dock score GLUT-4 (kcal/mol)
9-Octadecenoic acid (Z)-methyl ester	CCCCCCCCC=CCCCCCCCC(=O)OC	-4.2	-3.9
Eicosanoic acid	CCCCCCCCCCCCCCCCCCCC(=O)O	-4.9	-4.3
Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	CCCCCCCCCCCCCCCC(=O)OC(CO)CO	-5.9	-5.1
Octadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	CCCCCCCCCCCCCCCCCC(=O)OC(CO)CO	-6.3	-6.9
Methanecarbothiolic acid	CC(=O)S	-5.7	-5.7
4,5-Diamino-2-hydroxypyrimidine	C1=NC(=O)NC(=C1N)N	-5.7	-4.8
3-Deoxy-d-mannoic lactone	C1C(C(OC(=O)C1O)CO)O	-6.0	-5..8
1,3-Dioxane 2-methyl-	CC1OCCCO1	-3.7	-4.9
Tetradecanoic acid	CCCCCCCCCCCCCC(=O)O	-	-
3-Methyl-1-adamantaneacetic acid	CC12CC3CC(C1)CC(C3)(C2)CC(=O)O	-	-
n-Hexadecanoic acid	CCCCCCCCCCCCCCCC(=O)O	-	-
9,17-Octadecadienal (Z)-	C=CCCCCCCC=CCCCCCCCC=O	-6.4	-6.6
9,12-Octadecadienoic acid methyl ester	CCCCCC=CCC=CCCCCCCCC(=O)OC	-6.6	-6.6
9-Octadecenoic acid (Z)- methyl ester	CCCCCCCCC=CCCCCCCCC(=O)OC	-5.4	-4.6
Pentadecanoic acid methyl ester	CCCCCCCCCCCCCCC(=O)OC	-	-
Octadecanoic acid	CCCCCCCCCCCCCCCCCC(=O)O	-	-

Ligand	Canonical smiles	Dock score PPAR Gamma (kcal/mol)	Dock score GLUT-4 (kcal/mol)
9,12-Octadecadienoic acid (Z,Z)-	CCCCCC=CCC=CCCCCCCCC(=O)O	-4.5	-5.0
Eicosanoic acid	CCCCCCCCCCCCCCCCCCCC(=O)O	-5.1	-4.0
Naphthalene, 1,2,3,4-tetrahydro-1-methoxy	COC1CCCC2=CC=CC=C12	-5.0	-4.3
3-Phenylbut-1-ene	CC(C=C)C1=CC=CC=C1	-4.2	-4.4
Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	CCCCCCCCCCCCCCCC(=O)OC(CO)CO	-4.1	-4.2
1,2-Benzenedicarboxylic acid, diisooctyl ester	CC(C)CCCCCOC(=O)C1=CC=CC=C1C(=O)OCCCCCC(C)C	-4.3	-4.3

Supplementary Table S4: Pharmacokinetic properties.

ADME/T properties													
Compound	MW (g/mol)	Rotor	LogP	LogBB	Lipinski RO5	Absorption	LogS	accptHB	donorHB	TPSA	log Kp (cm/s)	BA	SA
Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	330.5	18	4.5	Yes	0	High	-4.57	4	2	66.76	-3.96	0.55	3.35
Octadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	358.56	20	4.89	No	0	High	-5.29	4	2	66.76	-3.36	0.55	3.58
Methanecarbothiolic acid	76.12	0	1.12	No	0	High	-0.5	1	0	55.87	-6.55	0.55	1.1
4,5-Diamino-2-hydroxypyrimidine	126.12	0	-0.09	No	0	High	-0.08	2	3	97.79	-8.24	0.55	1.81
3-Deoxy-d-mannoic lactone	162.14	1	0.72	No	0	High	-0.11	5	3	86.99	-8.04	0.55	3.12
9,17-Octadecadienal (Z)-	264.45	15	4.41	No	1	High	-4.7	1	0	17.07	-3.16	0.55	3.13
9,12-Octadecadienoic acid methyl ester	294.47	15	4.61	No	1	High	-4.97	2	0	26.3	-3.25	0.55	3.18
Pentadecanoic acid methyl ester	256.42	14	4.38	Yes	1	High	-4.82	2	0	26.3	-3.01	0.55	2.42
Octadecanoic acid	284.48	16	4.3	No	1	High	-5.73	2	1	37.3	-2.19	0.85	2.54

Minimal Ranges: MW=Molecular Weight (130.0/725.0), Rotor=No. of Rotatable Bonds (0.0/15.0), logP=log P for octanol/water (-2.0/6.5), logBB=log BB for brain/blood (Low), logS=log S for aqueous solubility (-6.5/0.5), Absorption (High), Lipinski Rule of 5 (RO5) Violations (max 4), accptHB=Acceptor-Hydrogen Bonds (2.0/20.0), donorHB=Donor- Hydrogen Bonds (0.0/6.0), log Kp=log Kp for skin permeability (Kp in cm/s), TPSA=topological polar surface area (0.0/450.0), BA=Bioavailability (0.00/0.55), SA=Synthetic accessibility (2.0/3.0).

Supplementary Table S5: Pharmacokinetic properties.

Ligand	Formula	MW	#Rotatable bonds	#H-bond acceptors	#H-bond donors	TPSA	iLOGP	ESOL Log S	GI absorption	BBB permeant	log Kp (cm/s)	Lipinski #violations	Bioavailability Score	Synthetic Accessibility
9-Octadecenoic acid (Z)-methyl ester	$C_{19}H_{36}O_2$	296.49	16	2	0	26.3	4.75	-5.32	High	No	-2.82	1	0.55	3.16
Eicosanoic acid	$C_{20}H_{40}O2$	312.53	18	2	1	37.3	4.56	-6.44	Low	No	-1.61	1	0.85	2.77
Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5	18	4	2	66.76	4.5	-4.57	High	Yes	-3.96	0	0.55	3.35
Octadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{42}O_4$	358.56	20	4	2	66.76	4.89	-5.29	High	No	-3.36	0	0.55	3.58
Methanecarbothiolic acid	C_2H_4OS	76.12	0	1	0	55.87	1.12	-0.5	High	No	-6.55	0	0.55	1.1
4,5-Diamino-2-hy droxypyrimidine	$C_4H_6N_4O$	126.12	0	2	3	97.79	-0.09	-0.08	High	No	-8.24	0	0.55	1.81
3-Deoxy-d-mannoic lactone	$C_6H_{10}O_5$	162.14	1	5	3	86.99	0.72	-0.11	High	No	-8.04	0	0.55	3.12
1,3-Dioxane 2-methyl-	$C_5H_{10}O_2$	102.13	0	2	0	18.46	1.79	-0.84	High	No	-6.5	0	0.55	2.28
Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	12	2	1	37.3	3.32	-4.31	High	Yes	-3.35	0	0.85	2.09
3-Methyl-1-adamantaneacetic acid	$C_{13}H_{20}O_2$	208.3	2	2	1	37.3	2.17	-3.25	High	Yes	-5.03	0	0.85	3.96
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	14	2	1	37.3	3.85	-5.02	High	Yes	-2.77	1	0.85	2.31
9,17-Octadecadienal (Z)-	$C_{18}H_{32}O$	264.45	15	1	0	17.07	4.41	-4.7	High	No	-3.16	1	0.55	3.13
9,12-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294.47	15	2	0	26.3	4.61	-4.97	High	No	-3.25	1	0.55	3.18
9-Octadecenoic acid (Z)- methyl ester	$C_{19}H_{36}O_2$	296.49	16	2	0	26.3	4.75	-5.32	High	No	-2.82	1	0.55	3.16
Pentadecanoic acid methyl ester	$C_{16}H_{32}O_2$	256.42	14	2	0	26.3	4.38	-4.82	High	Yes	-3.01	1	0.55	2.42
Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	16	2	1	37.3	4.3	-5.73	High	No	-2.19	1	0.85	2.54
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	14	2	1	37.3	4.14	-5.05	High	Yes	-3.05	1	0.85	3.1
Eicosanoic acid	$C_{20}H_{40}O_2$	312.53	18	2	1	37.3	4.56	-6.44	Low	No	-1.61	1	0.85	2.77
Naphthalene, 1,2,3,4-tetrahydro-1-methoxy	$C_{11}H_{14}O$	162.23	1	1	0	9.23	2.36	-2.74	High	Yes	-5.5	0	0.55	2.29
3-Phenylbut-1-ene	$C_{10}H_{12}$	132.2	2	0	0	0	2.4	-3.45	Low	Yes	-4.32	1	0.55	1.65
Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5	18	4	2	66.76	4.5	-4.57	High	Yes	-3.96	0	0.55	3.35
1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390.56	16	4	0	52.6	5.42	-6.66	High	No	-2.71	1	0.55	3.41

Minimal Ranges: MW=Molecular Weight (130.0/725.0), Rotor=No. of Rotatable Bonds (0.0/15.0), logP o/w=log P for octanol/water (-2.0/6.5), logBB=log BB for brain/blood (Low), logS=log S for aqueous solubility (-6.5/0.5), % Human Oral Absorption in GI (High), Lipinski Rule of 5 Violations (maximum is 4), accPthB=Acceptor-Hydrogen Bonds (2.0/20.0), donorHB=Donor-Hydrogen Bonds (0.0/6.0), log Kp=log Kp for skin permeability (Kp in cm/s), TPSA=topological polar surface area (0.0/450.0), Bioavailability=(0.00/0.55), Synthetic accessibility=(2.0/3.0).