Pharmacodynamic Interaction of *Salacia chinensis* with Saxagliptin in Normal and Diabetic Rats

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Abstract:

This study investigated the potential pharmacodynamic interaction between *Salacia chinensis*, a widely used antidiabetic agent in Ayurveda, and saxagliptin, a commonly used DPP-4 inhibitor. The preliminary in vitro studies indicated α-amylase and α-glucosidase inhibitory potential of Salacia chinensis hydro-ethanolic extract (SCHE). Further studies in 3T3-L1 adipocytes indicated a dose-dependent increase in glucose uptake, indicating the insulin-sensitizing effect of SCHE at 50 and 100 µg/ml doses. In vivo dose optimization was conducted in normal Wistar rats by evaluating serum glucose levels following administration of SCHE at 100 and 200 mg/kg. Based on the dose-dependent hypoglycaemic effect, 200 mg/kg was selected for further studies. Combined administration of saxagliptin along with SCHE produced significantly higher percentage reduction in blood glucose levels as compared to the saxagliptin only group in normal animals. Diabetes was induced in rats by feeding them with a high-fat (60%) diet for 28 days with streptozotocin (35 mg/kg) intraperitoneal administration on day 14, and animals were treated orally with SCHE, saxagliptin (1 mg/kg), or their combination for 28 days. Pharmacodynamic interactions were evaluated on day 29 by measuring serum glucose and glycated haemoglobin levels. Study results indicated antidiabetic potential of the SCHE treatment, which attenuated the blood glucose, triglyceride, total cholesterol, LDL cholesterol levels. The SCHE treatment also enhanced glucose sensitivity as determined from the oral glucose tolerance test (OGTT) and enhanced insulin sensitivity as observed from the insulin tolerance test and HOMA-IR values. Combination of saxagliptin and SCHE demonstrated significantly higher amelioration of these parameters in diabetic animals as compared to the saxagliptin only group, which might be arising from the significant pharmacodynamic interaction of saxagliptin with SCHE. Therefore, caution is advised when co-administering these agents, as saxagliptin dose adjustments may be necessary in clinical settings to prevent severe hypoglycaemia.

Keywords:

Diabetes mellitus, Drug-herb interaction, Pharmacodynamic interaction, *Salacia chinensis*, saxagliptin,

1.0. Introduction

Non-communicable diseases (NCD) are responsible for approximately 71% of global deaths, as per World Health Organization (WHO) reports. Diabetes mellitus (DM) is the prominent single disease, contributing to a higher number of deaths caused by NCDs. Diabetes mellitus is considered a global epidemic due to its increased prevalence and its effect on public health and quality of life worldwide[1-2]. It is a rapidly increasing long-term metabolic condition marked by elevated blood glucose levels resulting from either diminished insulin secretion resulting from pancreatic beta cell damage (Type I Diabetes mellitus) or it might be caused due to insulin resistance affecting predominantly insulin-sensitive tissues liver, adipose tissue, and skeletal muscles (Type II Diabetes mellitus)[3]. It significantly affects the health and quality of life of the individual impacted. The occurrence of diabetes is increasing at an alarming rate worldwide, rising from 200 million people in 1990 to 830 million in 2022. Diabetes is associated with microvascular and macrovascular complications, causing kidney failure, blindness, limb amputations, stroke, and heart failure. Hyperglycaemia caused by diabetes was the causative factor for 11% cardiovascular deaths and approximately 2 million deaths by renal dysfunction in 2021, and it has directly caused 1.6 million deaths globally[2].

Diabetes is considered a lifestyle disorder, which can be prevented and treated by modifying lifestyle behaviors, such as inclination towards a healthy diet with reduced calorie intake, an increase in physical activity with a minimum of 150 exercise hours per week, and avoiding predisposing factors such as consumption of alcohol and tobacco. Pharmacological management of diabetes includes the administration of insulin analogues and oral antidiabetic agents such as metformin, sulfonylureas, thiazolidinediones, alpha-glucosidase inhibitors, dipeptidyl peptidase (DPP)-4 inhibitors, sodium-glucose cotransporters type 2 inhibitors, and glucagon like peptide (GLP)-1 receptor antagonists[4-5]. Among various antidiabetic agents, metformin is the first-line drug used either alone or in combination with other agents. It is highly tolerated with the pleiotropic mechanism of action. Dipeptidyl peptidase (DPP)-4 inhibitors are incretin-based therapies that produce effects similar to incretins physiologically. DPP-4 inhibitors cause inhibition of this enzyme, reducing the breakdown of incretins GIP and GLP-1, further causing reduced glucagon secretion and enhanced secretion of insulin from pancreatic beta cells. Due to their lower adverse effects profile, these drugs are currently considered second-line drugs for the treatment of T2D for treatment of patients with metformin resistance. Approved drugs in this class include alogliptin, linagliptin, saxagliptin, sitagliptin, and vildagliptin[6]. Among all the DPP-4 inhibitors, saxagliptin is the widely used agent due to

its slower dissociation from the enzyme, thus causing inhibition for longer times thus producing better management of serum glucose levels as compared to other agents[7].

The chronic nature of diabetes mellitus and the adverse effect profile of antidiabetic agents have led to increased usage of the herbal products from the traditional systems of medicine. There is currently a list of 400 medicinal plants enlisted with the World Health Organization with antidiabetic properties[8-9]. These medicinal plants are rich in phytoconstituents that act via multiple mechanisms and are considered to be safe with a limited adverse effect profile. These beneficial effects of medicinal plants led to increased inclination of patients towards traditional systems of medicine such as Ayurveda, which is causing a surge in therapeutic usage of these medicinal plants in chronic diseases such as diabetes mellitus[10]. Ayurveda is the traditional system of medicine practised in India for many centuries. *Salacia chinensis*, known as Saptrangi in Ayurveda. It is an important medicinal plant in Ayurveda, traditionally used for managing a range of metabolic and inflammatory disorders such as diabetes mellitus (Madhumeha), and associated conditions such as obesity, hyperlipidemia, rheumatism, skin diseases, and digestive disturbances. The major phytoconstituents identified in this plant are polyphenols such as mangiferin, sulfated thiosugars such as salacinol, salaprinol, flavonoids, such as quercetin, gallic acid, kampferol, and other chemical classes include lignans, glycosides, and terpenes. Pharmacological studies reported antidiabetic, anti-obesity, antihyperlipidemic, nephroprotective, hepatoprotective, anticancer, antihypertensive, antifibrolytic, and immunomodulatory potential of *Salacia chinensis* in preclinical studies[11]. The antidiabetic potential of *Salacia chinensis* roots, stem, and its individual phytoconstituents was evaluated, and studies reported their α-glucosidase, α-amylase inhibitory, and antidiabetic activity[12]. The increased usage of medicinal plants from traditional systems of medicine by diabetic patients might cause drug-herb interactions of the antidiabetic drugs with the medicinal plant, which might cause adverse effects and toxicity. In this scenario, the current study aimed to evaluate the pharmacodynamic interaction of the DPP-4 inhibitor saxagliptin with widely used antidiabetic Ayurvedic drug *Salacia chinensis* in type 2 diabetic rat model.

2.0. Materials and Methods:

2.1. Materials

Saxagliptin was provided by MSN Pharmaceuticals (Hyderabad, India) as a gift sample. The 3T3-L1 preadipocyte cell line was procured from the American Type Culture Collection

(Manassas, USA). Other cell culture consumables, such as Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum, penicillin-streptomycin antibiotic cocktail, and trypsin-EDTA solution, were procured from Gibco Life Technologies (Massachusetts, USA). The chemicals utilized for differentiation of the pre-adipocytes: Insulin, dexamethasone, 3-Isobutyl-1-methylxanthine, and MTT reagent for cytotoxicity estimation, streptozocin for induction of Type 2 Diabetes mellitus, were procured from Sigma-Aldrich (St. Louis, MO, USA). The glucose uptake assay kit was procured from Abcam (Cambridge, USA). Glucose oxidase-peroxidase (GOD-POD) kit, triglyceride kit, Insulin kit, total cholesterol, HDL cholesterol, LDL cholesterol kit were procured from Accurex (Mumbai, India). High fat diet (60%) was procured from the National Institute of Nutrition (Hyderabad, India). Salacia chinensis extract was procured from

2.2. Preparation of the extract

The whole plant of *Salacia chinensis* was shade-dried, ground, and sieved to collect fine powder. Hundred grams of plant material powder was taken into five conical flasks, and 250 ml of 70% ethanol was added to the flask and extracted using a wrist shaker with continuous shaking for 12 h, followed by allowing the flask to stand for 8 hours. The extracts obtained were subjected to filtration using Whatman filter paper (0.22 µm membrane), and fresh solvent was added for further extraction of the residual plant material. Further solvent enriched with *Salacia chinensis* was removed under reduced pressure using rotary evaporator (Buchi, Mumbai, India) to avoid degradation of thermolabile constituents in the extract. The extract was then subjected to freeze-drying using a freeze dryer. Extraction efficiency (EE) of the extracts was calculated using the formula

EE = Amount of extract/Amount of plant material X 100.

The *Salacia chinensis* whole plant hydroethanolic extract (SCHE) was dissolved in DMSO at a concentration of 100 mg/ml (stock solution) by vortex mixer for 15 minutes, centrifuged at 8000 rpm for 10 minutes, and the supernatant obtained was filtered through a 0.22 µm membrane filter. Different concentrations of the extracts (1.56, 3.125, 6.25, 12.5, 25, 50, and 100 µg/ml) were prepared from the stock solution by diluting it in DMEM media as per the experiment and used as treatments in experimental procedures.

2.3. Cell viability assay for SCHE extract in 3T3-L1 Cells

3T3-L1 cells are pre pre-adipocyte cell line utilized for evaluating the effect of SCHE extract on glucose uptake and thereby causing any pharmacodynamic interaction with Saxagliptin. Before this the cytotoxicity assay for SCHE extract was performed in 3T3-L1 cells using the MTT assay**.** Cell viability assay was performed using slightly modified Kang et al., method. Pre-adipocyte 3T3-L1cells were plated at the density of 5,000/well in a 96 micro plate well in maintenance media (DMEM media supplemented with 10% FBS, 1% streptomycin-gentamycin) and allowed to grow in a humidified atmosphere containing 5% CO_2 at 37°C. After 24 h cells were treated with 0 (0.1% DMSO in DMEM media), 6.25, 12.5, 25, 50 and 100 µg/ml concentrations of all the extracts and maintained under same conditions for 48h. Post incubation, media containing different concentrations of extracts was removed and 100 µL of MTT solution in DMEM media (5 mg/ml) was added to each well and further incubated for 4 h at 37°C. Medium containing MTT was removed and osazone crystals that were formed in each well were solubilized in DMSO for 30 minutes under dark conditions and absorbance was measured with Spectramax M4 multidetection plate reader (California, USA) at 570 nm . In this assay three wells were not seeded with cells, which acted as blank, absorbance values of all treatment groups were subtracted with blank absorbance value to nullify background absorbance[13]. The wells which were treated with zero concentration of extract were considered as normal control (100% viability) and percentage cell viability in treatment groups were calculated using below formula

$$\text{Percentage cell viability} = \text{Absorbance}_{\text{treatment}} / \text{Absorbance}_{\text{control}} \times 100$$

2.4. Glucose uptake assay

The effect of SCHE extract on glucose uptake in to adipocytes was estimated using Abcam colorimetric Glucose uptake assay kit (Cambridge, UK). In this method uptake of glucose analog 2- deoxy glucose (2-DG) in to adipocytes was measured using NADPH amplifying system. For this assay 3T3-L1 preadipocytes were seeded in to 96 well plate and differentiated to mature adipocytes. Briefly, the procedure for differentiation is Preadipocyte 3T3-L1 cells were plated at 1 x 10^4 cells per well in 24 well plate and allowed to grow till confluency (48h) in maintenance media in a humidified atmosphere containing 5% CO_2 at 37°C. For differentiation of preadipocyte cells, they were stimulated post confluent with a differentiation cocktail I (Day 0) containing 1 µg/mL insulin, 0.25 µM dexamethasone and 0.5 mM IBMX in maintenance media for 2 days. Cells were further incubated with differentiation cocktail II (Day 2) containing maintenance media with 1 µg/mL insulin for 2 more days. After incubation

with differentiation cocktail II medium, it was replaced with maintenance media (Day 4) for every 2 days interval till day 8.

After differentiation cells were overnight serum starved, washed with PBS and then incubated for 40 minutes with KRPH buffer containing 2% BSA. Cells were further treated for 2 h with SCHE treatments (50 & 100 µg/mL) and positive standard insulin (1 µM). Subsequently, cells were incubated with low glucose DMEM containing 10 mM 2-DG for 20 minutes, washed with PBS and lysed with 80 µL of extraction buffer. Blank cells were incubated with low glucose DMEM without 2-DG, and cell lysate was prepared. Cell lysates were freeze-thawed once, heated at 85 °C for 40 minutes and cooled on ice for 5 minutes. Samples were neutralized by adding of 10 µL of neutralization buffer, centrifuged at 500 rpm for 1-2 minutes, and the supernatant was collected. 50 µL of blank, NC, DC, treatments and insulin groups supernatant and standard samples (0-100 pM) were added to 96 well plate. Subsequently reaction mixture containing 8 µL of assay buffer and 2 µL of enzyme mix was added to each well and incubated at 37°C for 1 h. Further samples were subjected to extraction and neutralization for degrading unused NADP. To these samples, 38 µL of reaction mixture B comprising 20 µL of glutathione reductase, 16 µL of substrate, and 2 µL of recycling mix was added. Absorbance of all the samples was measured in kinetic mode at 412 nm for every 120 seconds until the absorbance of the highest standard reaches 1.5. 2-DG concentration calculated from a standard curve and normalized with its protein content.

2.5. Enzymatic Inhibitory Activity

The SCHE extract was evaluated for its inhibitory effect on alpha-glucosidase, alpha-amylase, and DPP-4 activity in the concentration range of 0.78 to 800 µg/ml. The α-glucosidase inhibitory activity was determined following the method of Pandithurai et al. (2015). A reaction mixture was prepared by adding 1 mL of 2% (w/v) sucrose solution in 0.2 M Tris buffer (pH 8.0) to 1 mL of SCHE extract at various concentrations (0.78–800 µg/mL). The mixture was incubated at room temperature for 5 minutes, followed by the addition of 1 mL α-glucosidase enzyme (10 U/mL) to initiate the reaction. The mixture was then incubated at 35 °C for 40 minutes. The reaction was terminated by adding 2 mL of 6N HCl. The absorbance was measured at 540 nm using a spectrophotometer. Acarbose served as the standard drug control. The α-amylase inhibitory activity was assessed according to the method of González-Montoya et al. (2018). The reaction mixture consisted of 1 mL of SCHE extract (0.78–800 µg/mL) and 1 mL of α-amylase solution (2 U/mL), which was pre-incubated at 37 °C for 30 minutes. Following this, 1 mL of starch solution was added and the reaction continued for another 10

minutes at 37 °C. The reaction was stopped by adding 1 mL of DNS reagent (prepared with 12.0 g sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM of 3,5-dinitrosalicylic acid). The mixture was boiled for 5 minutes. A negative control (without sample) and acarbose (as positive control) were included. Absorbance was measured at 540 nm. DPP-4 inhibitory activity was evaluated using a DPP-4 Inhibitor Screening Kit (Cayman Chemical, Ann Arbor, MI), based on a fluorescence assay. In a 96-well plate, 10 µL of SCHE extract (in DMSO) at various concentrations was mixed with 30 µL of diluted assay buffer, 10 µL of diluted human-recombinant DPP-4 enzyme, and 50 µL of diluted fluorogenic substrate (Gly-Pro-AMC). Negative control wells contained solvent only, while sitagliptin was used as the positive control. The plate was shaken gently and incubated at 37 °C for 30 minutes. Fluorescence was measured using a microplate reader with excitation at 350–360 nm and emission at 450–465 nm[14]. The percentage of inhibition was calculated using the formula. The IC_{50} of this enzymatic inhibitory activity of the extract was performed using Graph Pad Prism version 7.0.

$$\% \text{ Inhibition} = (A_{Control} - A_{sample}/A_{Control}) * 100$$

2.6. Animals and Study Design

Healthy male Wistar rats (8 weeks old) were procured from Mahaveer Enterprises (Hyderabad, India) and acclimatized under standard laboratory conditions (12 h light/dark cycle, 40–60% humidity, 22±3°C) for one week, with free access to water and a standard pellet diet (Hindustan Lever Ltd., Bangalore, India). Experimental groups consisted of six rats each to ensure statistical significance. Saxagliptin was dissolved in water, while SCHE was freshly suspended in 0.1% carboxymethyl cellulose before administration.

2.7. Pharmacodynamic Interaction Study in Normal Animals

The experimental animals were divided into four groups, each containing six animals. The animals were administered treatments as per their group, consecutively, for 3 days, followed by measurement of glucose at 0, 1, 2, 4, 6, 8, 10, and 12 h post administration of the last dose. The details of the experimental groups are provided below

- Group I – Saxagliptin group: Animals were orally administered with 1 mg/kg of Saxagliptin solution for 3 days
- Group II –SCHE low dose group: Animals were orally administered with 100 mg/kg of SCHE in 0.1% CMC for 3 days

- Group III –SCHE high dose group: Animals were orally administered with 200 mg/kg of SCHE in 0.1% CMC for 3 days

- Group IV –Combination group: Animals were orally administered with 1 mg/kg of Saxagliptin, followed by SCHE at 200 mg/kg simultaneously with an interval of five minutes

2.8. Pharmacodynamic Interaction Study in Diabetic Animals

Type 2 Diabetes mellitus was induced in the experimental animals by providing a high-fat diet (60%) to the experimental animals for 28 days with intermittent injection of low-dose streptozotocin (STZ) intraperitoneally. After the acclimatization period, animals were randomly divided into the following groups according to their treatment. All the experimental animals except the normal control were fed with a high-fat diet (60%) procured from the National Institute of Nutrition, Hyderabad. The composition of the high-fat diet is provided in Table no.3. The experimental animals in the normal control group were fed with normal chow comprising 10% fat. Experimental animals were administered with low dose (35 mg/kg) of streptozocin on day 14. For this, animals fasted overnight before induction of DM with STZ. As STZ is unstable and light sensitive, all related preparations were performed in aluminium foil-covered tubes. Just prior to injection, STZ solution was prepared in sodium citrate buffer (pH 4.5) to obtain a strength of 30 mg/ml. It was maintained at refrigerated temperatures throughout administration by keeping the STZ containing vial in an ice box, and injections to all the animals were performed within 15 minutes to avoid degradation. STZ was injected intraperitoneally at a 35 mg/kg body weight dose to induce DM in animals. Dextrose solution (20%) was administered intraperitoneally 4-6 h post-STZ administration to overcome possible initial hypoglycaemia, and animals were further provided till 24 h with 50% dextrose water. Induction of DM was verified 72 h post administration of STZ by serum glucose determination[15]. The experimental design consisted of the following groups.

- Group I – Normal Control group: Animals fed with a normal diet and orally administered with 0.1% CMC for 28 days, and normal saline on day 14

- Group II –Disease control group: Animals were fed with a high-fat diet (60%) and orally administered with 0.1% CMC for 28 days and a low dose (30 mg/kg) STZ on day 14

- Group III –Saxagliptin group: Animals were fed with a high-fat diet (60%) and orally administered with saxagliptin at 1 mg/kg for 28 days, and a low dose (30 mg/kg) of STZ on day 14

- Group III –SCHE group: Animals were fed with a high-fat diet (60%) and orally administered with SCHE extract at 100 mg/kg for 28 days, and a low dose (30 mg/kg) of STZ on day 14

- Group IV - Co-administration group: Animals were fed with a high-fat diet (60%) and orally administered with saxagliptin at 1 mg/kg followed by 100 mg/kg SCHE extract for 28 days, and a low dose (30 mg/kg) STZ on day 14

The pharmacodynamics interaction studies were carried out at the end of the experimental period in diabetic animals. The animals were fasted overnight on day 28, and blood was collected on day 29 for determining serum glucose, insulin, HbA1c, triglyceride, total cholesterol, LDL, and HDL cholesterol levels. The effects of treatments on glucose sensitivity was determined by oral glucose tolerance test was performed on day 22, and for determining the insulin sensitivity Intraperitoneal Insulin Tolerance Test (ITT) was performed on day 24. For insulin sensitivity determination along with ITT, HOMA-IR was also determined at the end of the study.

2.9. Oral Glucose Tolerance Test (OGTT)

The OGTT test was carried out on day 21 of the study, before the test animals were fasted overnight. Glucose was administered orally to the animals at a concentration of 2 g/kg dissolved in distilled water. At time points 0, 0.25, 0.5, 1, 1.5, and 2h, blood (20 µL) was collected through the tail vein in restrained animals. Serum samples were collected by centrifugation of blood samples at 5000 rpm for 5 minutes at 4°C. Serum glucose levels were determined using the GOD-POD method. The serum glucose concentration versus time graph was plotted, and areas under the curves (AUC) were calculated using the trapezoidal method[16].

2.10. Insulin tolerance test (ITT)

Insulin sensitivity of the animals was determined by performing the ITT test on day 24 of the study. Similar to OGTT, animals were fasted overnight before performing this test and intraperitoneally administered insulin (0.75 IU/kg). At time points 0, 0.25, 0.5, 1, 1.5, and 2h, blood (20 µL) was collected through the tail vein in restrained animals. Serum samples were collected by centrifugation of blood samples at 5000 rpm for 5 minutes at 4°C. Serum glucose levels were determined using the GOD-POD method. Plasma glucose concentration versus time graph was plotted to observe reduction in glucose concentration in all the experimental groups as time progresses[15].

2.11. Biochemical parameter analysis

After completion of the 28-day study period, all animals were fasted overnight and sacrificed after collection of whole blood from retro-orbital plexus puncture under mild anaesthesia. Serum samples were separated by centrifuging blood samples at 5000 rpm for 5 minutes at 4°C. From these serum samples following parameters were determined.

2.11.1. Estimation of Serum Glucose Levels

Serum glucose determination for all the experiments was performed using the GOD-POD method based glucose assay kits (Tulip diagnostics) as per the provided protocol. To a micro centrifuge tube, 1 ml of working reagent, 100 µL of test sample or standard, or distilled water (blank) was added, and absorbance was measured at 505 nm after incubation of samples at 37°C for 10 minutes. Glucose levels of test samples were measured from below formula

2.11.2. Estimation of Serum HbA1c Levels

Ion exchange resin method-based kit was used for the measurement of HbA1c levels in the blood samples as per protocol. Hemolysate of the samples and control (provided in kit) was prepared by adding 500 µL of lysis reagent to micro centrifuge tubes. These tubes were further added with 100 µL of reconstituted control and test samples and incubated for 5 minutes. Hemolysed samples and control (100 µL) were added to the ion exchange resin tubes with a resin separator, vortex mixed for 5 minutes, and allowed to stand for sedimentation of resin. Supernatant was collected using a resin separator and subjected to absorbance measurement at 415 nm for measurement of HbA1c in control and test samples. Total haemoglobin fraction was measured by adding 200 µL of hemolysate of samples and control from previous steps to 5 ml of distilled water, mixed well, and absorbance measured. Percentage HbA1c determined using below formulas

2.11.3. Estimation of Serum Insulin Levels & HOMA-IR

ELISA kit sensitive to rats was procured from Elabsciences (Texas, USA) and utilized for the measurement of insulin levels as per the provided protocol. The ELISA plate was incubated for 90 minutes at 37°C after the addition of the test sample or standards (100 µL) for binding of insulin to its antibodies. Further samples, standards were discarded, and biotinylated secondary antibody (100 µL) was added, and the reaction mixture was incubated for 60 minutes at 37°C. Post-incubation secondary antibody was discarded, washed, and incubated for 15 minutes at 37°C after the addition of HRP conjugate (100 µL) to the plate. Further reaction mixture was

discarded, washed, incubated with substrate (90 µL) for 15 minutes at 37°C, and absorbance was measured at 450 nm after addition of stop solution (50 µL). For estimation of homeostatic model assessment of insulin resistance (HOMA-IR).

2.11.4. Estimation of Serum Triglyceride Levels

Serum triglyceride estimation of samples was performed using the triglycerides assay kit (Tulip diagnostics) as per the provided protocol. In a micro centrifuge tube, 1 ml of working reagent and 100 µL of test sample or standard or distilled water (blank) was taken, well mixed, incubated for 05 minutes at 37°C, and absorbance was measured at 505 nm against the blank.

2.11.5. Estimation of Total Cholesterol Levels

Total cholesterol estimation of samples was performed using the total cholesterol assay kit (Tulip Diagnostics) as per the provided protocol. To a micro centrifuge tube, 1 ml of working reagent, 100 µL of test sample or standard, or distilled water (blank) was added, well mixed, incubated for 05 minutes at 37°C, and absorbance against blank was measured at 505 nm.

2.11.6. Estimation of LDL Cholesterol Level

LDL cholesterol (LDL-C) estimation of samples was performed using the LDL cholesterol kit (Tulip diagnostics) as per the provided protocol. To a micro centrifuge tube, 375 µL LDL-D reagent and 105 µL of test sample or standard were added, well mixed, and incubated for 05 minutes at 37°C. Post incubation step reaction mixture was incubated for 05 minutes at 37°C after addition of LDL-D reagent 2 (125 µL), and absorbance was measured against blank at 546 nm.

2.11.7. Estimation of HDL Cholesterol Level

HDL cholesterol (HDL-C) estimation of samples was performed using the HDL-C kit (Tulip diagnostics) as per the provided protocol. In a micro centrifuge tube, 100 µL of precipitating reagent, 100 µL of serum test sample were taken, well mixed, and incubated for 5 minutes at room temperature. Clear supernatant was collected from the samples after centrifugation at 3000 rpm. In a micro centrifuge tube, 1 ml of working reagent and 50 µL of supernatant or standard or distilled water (blank) were taken, vortexed, and incubated for 05 minutes at 37°C, and absorbance against blank was measured at 505 nm.

2.12. Statistical Analysis

The data in this study are expressed as Mean ± SD, and the results were analyzed using one-way or two-way analysis of variance based on the requirement using GraphPad Prism 9.01 software. Results with $p<0.05$ were considered as statistically significant

3.0 Results

3.1. SCHE Extract Preparation

The extraction of *Salacia chinensis* whole plant (SCHE) with hydro-ethanol (70:30) by the maceration method for 72 h, followed by the removal of solvent with rotary evaporation, produced an extract with a yield of 9.26%. The extract was further subjected to freeze-drying for the removal of residual solvent. The obtained extract was dissolved in Di-methyl sulfoxide (20 mg/ml), vortexed for 5 minutes, centrifuged at room temperature for 5 minutes at 8000 rpm, and the supernatant obtained was utilized as the stock solution for in vitro studies. The extract was suspended in 1% sodium carboxymethyl cellulose (100 mg/ml) and utilized for in vivo studies.

3.2. Effect of SCHE Extract on Cell Viability

Before determining the effect of SCHE extract treatment on glucose uptake, the noncytotoxic dose of this treatment has to be determined. MTT assay was performed to evaluate the possible cytotoxicity of the SCHE extract on 3T3-L1 preadipocytes in the concentration range of 6.25 to 100 µg/ml. There was no reduction in cell viability, or cytotoxicity was observed with SCHE treatment across the concentration range on 3T3L1 preadipocyte cells upon incubation for 48 h, as compared to the normal control cells treated with vehicle. The results of the cell viability assay are depicted in Figure 1. For the further glucose uptake assay, the highest non-cytotoxic concentrations of 50 and 100 µg/ml were used.

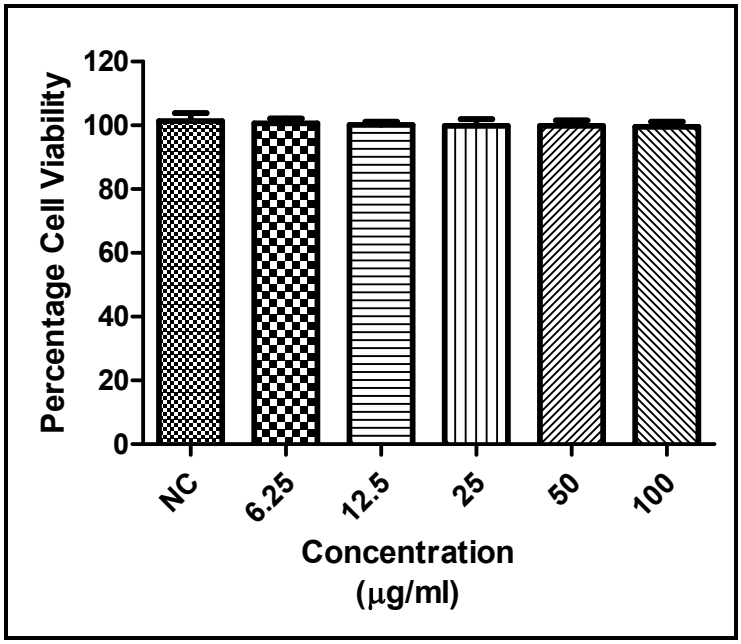

Figure 1. Effect of SCHE treatments on cell viability of 3T3-L1 preadipocyte cells. 3T3-L1 cells were seeded in 96 well plate at 5000 cells per well, and after overnight incubation, treated with different concentrations of SCHE for 48 hours. Cell viability was determined using the MTT assay. Data was represented as mean ± S.D.

3.3. Effect of SCHE Extract on Glucose Uptake

Glucose uptake is crucial for cellular glucose utilization and homeostasis of plasma glucose levels. Adipocytes are among the insulin-sensitive tissues. Insulin increases glucose uptake into the adipocytes and enhances adipogenesis in the adipocytes, thus reducing the blood glucose levels. Insulin resistance causes a reduction in glucose uptake, especially in adipose tissue, skeletal muscle, and liver, thereby enhancing the blood glucose levels. Differentiation of adipocytes caused a significant ($p<0.05$) increase in 2-DG uptake, which was 2.60 times higher than undifferentiated NC cells. Treatment of the differentiated adipocytes with positive control insulin significantly ($p<0.001$) enhanced glucose uptake by 2.83 times as compared to differentiated DC cells. Incubation of the differentiated adipocytes with SCHE has caused a non-significant increase in the glucose uptake by 1.38 times at 50 µg/ mL, and treatment with 100 µg/mL caused a significant increase ($p<0.001$) in glucose uptake by 1.94 times as compared to the DC cells. These results indicated the stimulatory effect of the SCHE treatments on the glucose uptake in differentiated adipocytes. The results are depicted in Figure 2.

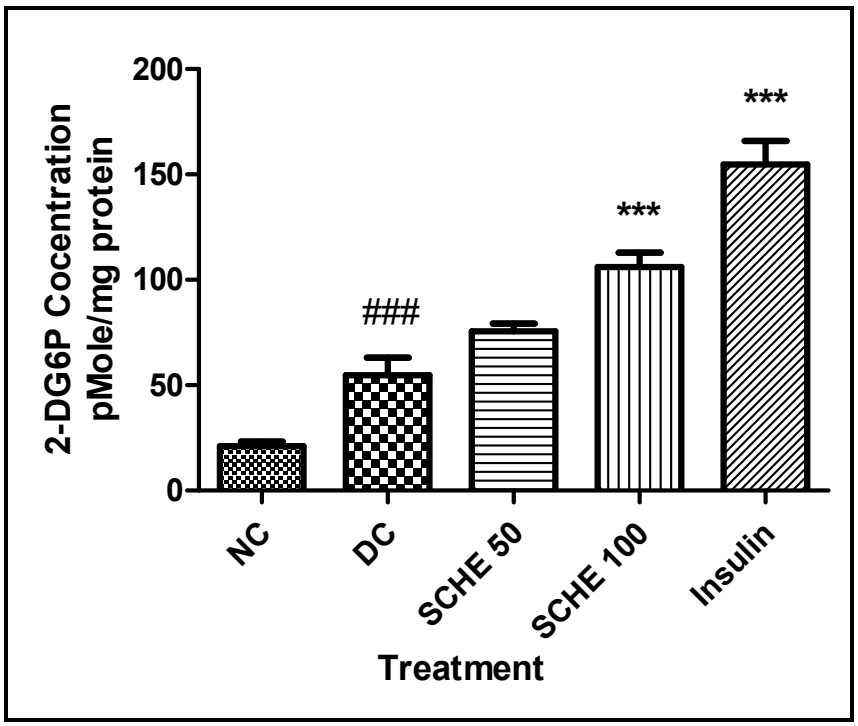

Figure 2. Effect of SCHE treatments on glucose uptake. 3T3-L1 cells were differentiated till day 8 and incubated with SCHE and insulin for 1 hour. Glucose uptake was determined by measuring intracellular 2-DG concentration and normalized with the concentration of protein. Insulin is used as a positive control in this assay. Data was represented as mean ± S.D, analysed by one-way ANOVA followed by Bonferroni post hoc test, and $p < 0.05$ was considered to be significant. ###$p < 0.001$ when compared to NC; ***$p < 0.001$ when compared to DC.

3.4. Effect of SCHE Extract on Enzymatic Inhibitory Activity

Alpha amylase and alpha-glucosidase are the enzymes that play a predominant role in the absorption of carbohydrates from the gastrointestinal tract. They cause breakdown of the complex polysaccharides into simpler and absorbable monosaccharides, thus increasing the blood glucose levels after food. DPP-4 is the enzyme that catalyzes the degradation of the incretins such as glucagon-like peptide (GLP)-I and gastric inhibitory peptide (GIP). Incretins are associated with pancreatic beta cell stimulation and cause the secretion of insulin after the ingestion of food. Inhibition of these enzymes reduces blood glucose levels, especially after ingestion of the food. The SCHE extract was subjected to evaluation of *in vitro* α-Amylase, α-Glucosidase, and DPP-4 inhibitory activities at concentrations ranging from 0.78 to 800 µg/ml. These studies indicated higher inhibitory activity of the SCHE extract on alpha-glucosidase with an inhibitory concentration (IC50) of 238.4 µg/ml and alpha-amylase with an IC50 of 291.52 µg/ml, whereas the extract demonstrated lower DPP-4 inhibitory activity with an IC50 of 527.39 µg/ml. The results are depicted in Figure 3.

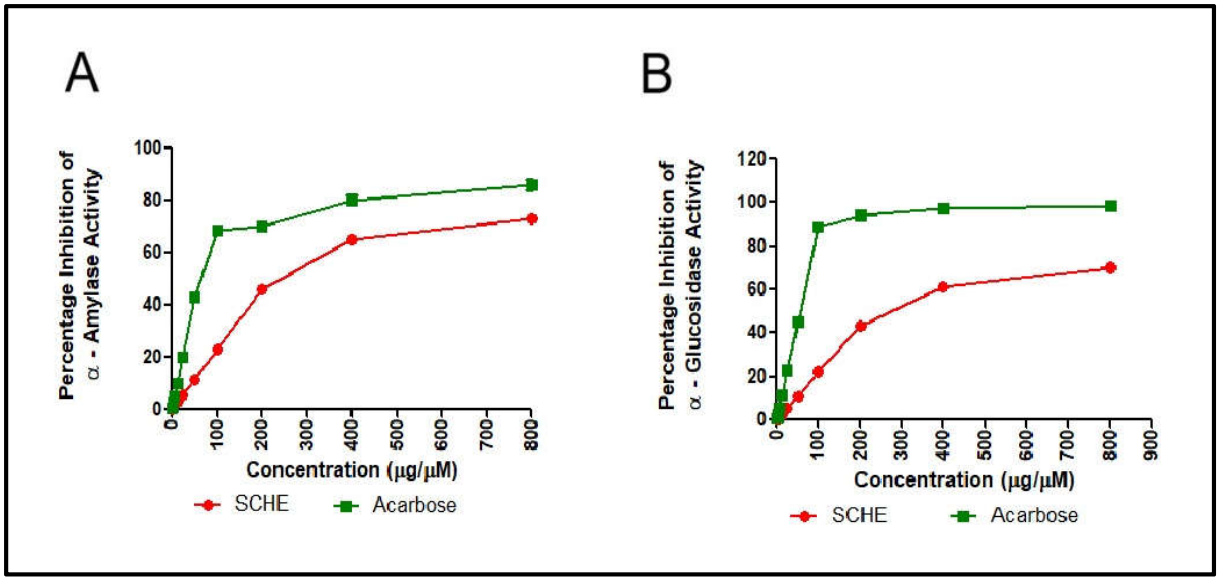

Figure 3. Effect of SCHE treatments on inhibition of in vitro α-amylase and α-glucosidase activity. Different concentrations of SCHE were incubated with α-amylase and α-glucosidase, and further incubated with respective substrates, sucrose and starch. After the addition of the stopping solution, the absorbance was measured, the percentage inhibition was determined, and the IC50 was calculated.

3.5. Pharmacodynamic Interaction Study in Normal Animals

The preliminary glucose-reducing effect of the SCHE treatment at two doses, 100 and 200 mg/kg, on normal animals was evaluated for selection of the SCHE dose for further studies and to determine the possible hypoglycaemic effect of the SCHE treatments. For this study, the experimental animals were treated for 3 days with respective SCHE treatments. There was a dose-dependent reduction in the blood glucose levels observed with the SCHE treatments at 100 and 200 mg/kg, with maximum percentage glucose reduction of 18.87% and 29.20% respectively, at the 3h time point. For further pharmacodynamic interaction studies, saxagliptin was dosed alone for a group of animals, and another group of animals were treated with SCHE at 200 mg/kg followed by saxagliptin. Administration of the saxagliptin to normal rats has caused a percentage reduction of blood glucose levels by 37.56% while co-administration of saxagliptin with SCHE caused a 45.13% reduction, which is significantly ($p<0.01$) higher than the saxagliptin alone group. The results are depicted in Table 1 and Figure 4.

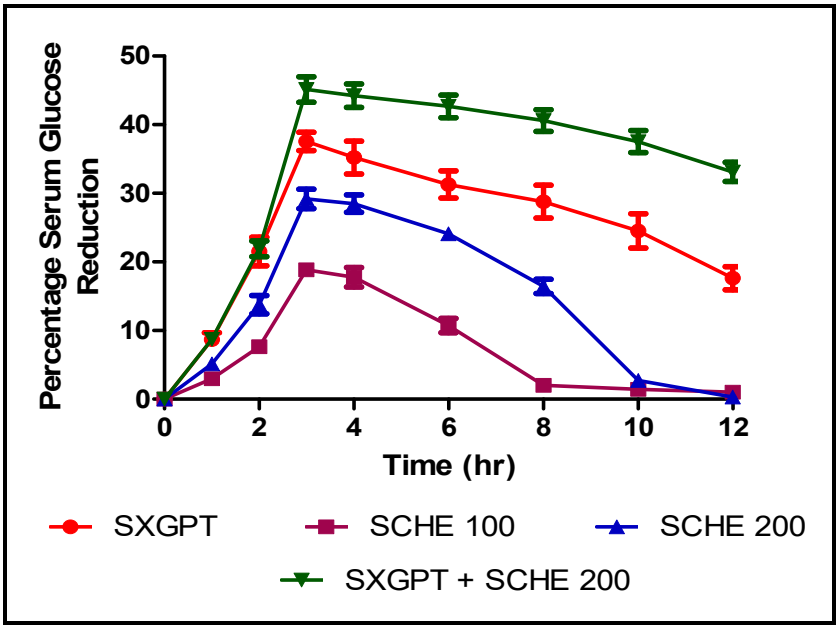

Figure 4. The effect of SCHE treatments on serum glucose levels and interaction with saxagliptin in normal animals. Experimental animals were divided into 4 groups with 3 animals per group. The animals were treated for 3 days with SCHE at 100 and 200 mg/kg, saxagliptin at 1 mg/kg, and saxagliptin along with SCHE at 200 mg/kg. On day 3, blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, and 12 h time intervals and serum glucose levels were measured.

Table 1. Effect of SCHE, saxagliptin, and combination treatment on blood glucose levels in normal animals

Time (h)	Serum Glucose levels (mg/dL)			
	Saxagliptin (1 mg/kg)	**SCHE (100 mg/kg)**	**SCHE (200 mg/kg)**	**Saxagliptin + SCHE (200mg/kg)**
0	90.15 ± 3.45	105.30 ± 2.14	98.10 ± 2.46	100.15 ± 4.26
1	82.31 ± 2.38	102.15 ± 2.09	93.05 ± 1.90	91.40 ± 0.95
2	70.75 ± 3.16	97.25 ± 1.78	84.59 ± 1.38	78.20 ± 1.25
3	**56.29 ± 1.64**	**85.43 ± 3.52**	**67.49 ± 1.53**	**54.95 ± 2.10**
4	58.41 ± 2.11	86.59 ± 0.87	68.19 ± 2.09	55.86 ± 1.31
6	61.94 ± 1.43	94.01 ± 2.21	72.48 ± 0.99	57.41 ± 2.01
8	64.20 ± 3.05	103.19 ± 2.05	81.97 ± 1.41	59.48 ± 1.58
10	68.04 ± 1.73	103.80 ± 1.57	95.43 ± 2.03	62.55 ± 1.72
12	74.26 ± 3.10	104.25 ± 2.48	97.78 ± 1.58	66.98 ± 1.59

3.6. Pharmacodynamic Interaction Study in Diabetic Animals

3.6.1. Effect of treatments on blood glucose and glycated haemoglobin levels

Hyperglycaemia is the characteristic feature of Diabetes mellitus. Feeding animals with a high-fat diet (60%) for 28 days with intermittent STZ administration (35 mg/kg intraperitoneal) on day 14 has caused the development of severe hyperglycaemia with fasting blood glucose levels ranging between 350-375 mg/dL, indicating the development of Diabetes mellitus. The composition of the high-fat diet is provided in the Table 2.

Table 2. Composition of a high-fat diet (60%) that was fed for experimental animals for the induction of type 2 diabetes

S.No.	Ingredient	Class	Weight (gm)	% Energy
1	Lactic casein	Protein	200	20
2	L- Cystine		03	
3	Sucrose	Carbohydrate	176.8	35
4	Lodex 10		125	
5	Corn starch		72.8	
6	Solka Floc	Fiber	50	45
7	Lard	Fat	245	
8	Soyabean oil		25	
9	S10026B	Mineral	50	NA
10	Choline bitartrate	Vitamin	02	
11	V10001C		01	

The fasting blood glucose level was significantly ($p<0.001$) higher in the disease control group as compared to the normal control group fed with a normal chow diet. Oral administration of the SCHE extract suspension at a 200 mg/kg dose for 28 days resulted in a significant ($p < 0.001$) reduction in fasting blood glucose levels, 1.76 times lower compared to the disease control animals, indicating the antidiabetic potential of the SCHE treatment. Administration of DPP-4 inhibitor saxagliptin to the respective group animals has caused a reduction in blood glucose level by 2.1 times as compared to the disease control group animals ($p<0.001$). Combination administration of the SCHE with Saxagliptin for 28 days has caused a significant reduction in the blood glucose levels by 2.51 times in comparison to the disease control group animals. The combination treatment of saxagliptin along with SCHE has caused a higher reduction in the blood glucose levels by 1.20 times, which is a significant ($p<0.05$) reduction in comparison to the saxagliptin alone administration. Similar to the fasting blood glucose levels, there was a significant increase in glycated haemoglobin levels observed in the disease control group as compared to the normal control, and treatments have ameliorated these levels significantly. This significantly higher reduction in the blood glucose levels and glycated haemoglobin levels in diabetic animals indicates possible interaction of the saxagliptin and SCHE extract when administered simultaneously. The results are depicted in figure 5.

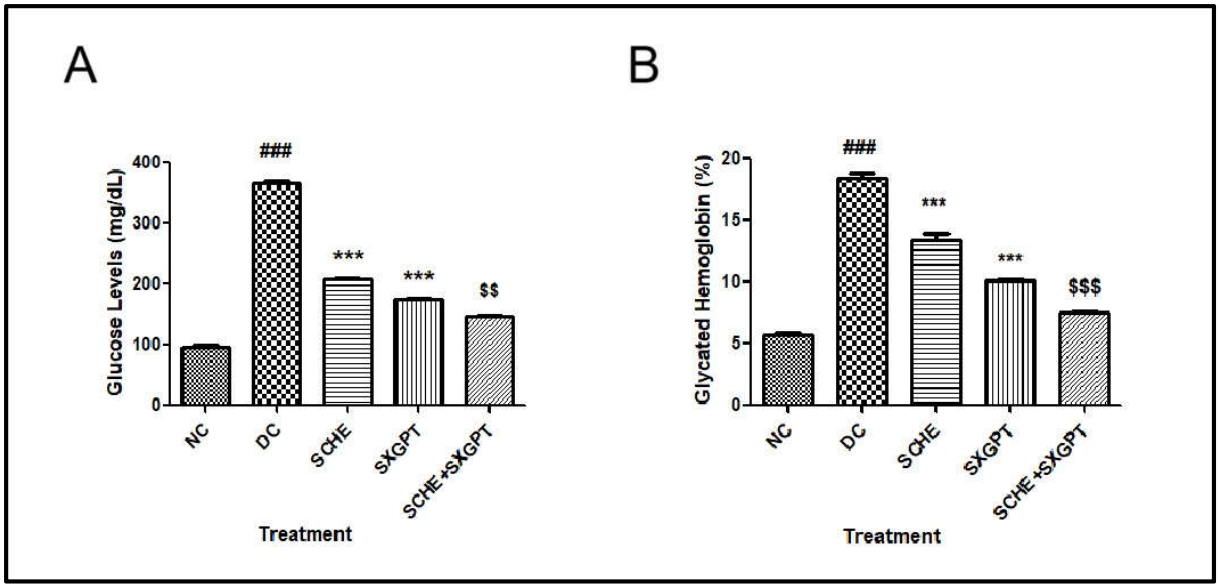

Figure 5. The effect of SCHE treatments on fasting blood glucose (A), glycated haemoglobin (B), and pharmacodynamic interaction of SCHE with saxagliptin in type 2 diabetic animals. The experimental animals were fed with a 60% high-fat diet for 28 days, and streptozocin (35 mg/kg) was administered on day 14 to induce type 2 diabetes. Experimental animals were subjected to their respective treatments of vehicle, SCHE (200 mg/kg), saxagliptin (1 mg/kg) and co-administration. On day 29, serum glucose and glycated haemoglobin levels were measured after overnight fasting. Data was represented as mean ± S.D, analysed by one-way ANOVA followed by Bonferroni post hoc test, and $p < 0.05$ was considered to be significant. ###$p < 0.001$ when compared to NC; ***$p < 0.001$ when compared to DC and $^{\$\$\$}p<0.001$ when compared to SXGPT.

3.6.2. Effect of treatments on glucose sensitivity

The oral glucose tolerance test is the method utilized for determining glucose sensitivity in clinical and preclinical settings, where glucose levels will be measured post oral glucose administration. Glucose administration by the oral route causes stimulation of the pancreatic beta cells and release of insulin, which acts on glucose absorbed and reduces its levels by acting on the insulin-sensitive tissues. Administration of the single dose STZ (35 mg/kg intraperitoneal) along with a high-fat diet for 28 days has caused a significant ($p<0.001$) increase in the area under curve of the glucose levels by 1.4 times as compared to the normal control animals. This increase in the area under curve for glucose in disease control animals indicates a reduction in glucose sensitivity in the animals after induction of diabetes mellitus. Oral administration of the SCHE extract suspension at a 200 mg/kg dose for 28 days resulted in a significant ($p < 0.01$) amelioration of glucose area under curve, 1.05 times lower compared to the disease control animals, indicating enhanced glucose sensitivity with the treatment. Administration of DPP-4 inhibitor saxagliptin to the respective group animals has caused a

reduction in glucose area under curve values by 1.25 times as compared to the disease control group animals ($p<0.001$). Combination administration of the SCHE with Saxagliptin for 28 days has caused a significant reduction in glucose area under curve values by 1.31 times in comparison to the disease control group animals. The combination treatment of saxagliptin along with SCHE has caused a higher reduction in the glucose area under curve values by 1.02 times, which is a significant ($p<0.05$) amelioration in comparison to the saxagliptin alone administration. This significantly higher attenuation of the glucose area under curve levels in diabetic animals indicates a significantly higher amelioration of the glucose sensitivity, which might be arising due to the interaction of the saxagliptin and SCHE extract when administered simultaneously. The results are depicted in Figure 6.

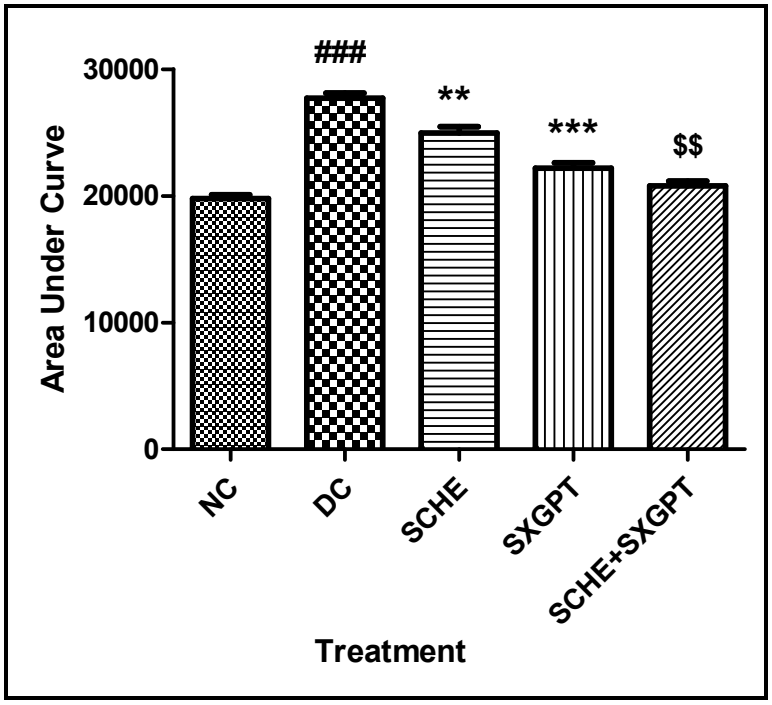

Figure 6. The effect of SCHE, saxagliptin, and combination treatments on Oral Glucose Tolerance Test (OGTT) in type 2 diabetic animals. The experimental animals were fed with a 60% high-fat diet for 28 days, and streptozocin (35 mg/kg) was administered on day 14 to induce type 2 diabetes. Experimental animals were subjected to their respective treatments of vehicle, SCHE (200 mg/kg), saxagliptin (1 mg/kg), and co-administration. On day 21, all the experimental animals were administered orally 2g/kg glucose after overnight fasting. Serum glucose levels were measured at 0, 0.25, 0.5, 1, 1.5, and 2h post glucose administration. Serum glucose versus time graph was plotted, and area under the curve for each animal was determined. Data was represented as mean ± S.D, analysed by one-way ANOVA followed by Bonferroni post hoc test, and $p < 0.05$ was considered to be significant. ###$p < 0.001$ when compared to NC; ***$p < 0.001$, **$p < 0.01$ when compared to DC and $^{\$\$\$}p<0.001$ when compared to SXGPT.

3.6.3. Effect of treatments on insulin sensitivity

Insulin resistance is the predisposing factor for the development of Type 2 diabetes mellitus. Insulin sensitivity of the animals can be evaluated using insulin tolerance test, where glucose levels will be monitored after administration of insulin to the experimental animals. Along with the insulin tolerance test, HOMA-IR is also an indicator of insulin sensitivity, which is considered an indirect indicator of insulin sensitivity. In the insulin tolerance test, there was a slight reduction in glucose levels after insulin administration in the disease control group observed, whereas the reduction was higher in the normal control group, indicating the tolerance nature of the diabetic animals towards insulin. There was an improvement in insulin tolerance with a greater decrease in glucose levels in the treatment groups SCHE, saxagliptin alone, and combination of saxagliptin with SCHE as compared to disease control. There was a higher reduction in blood glucose levels in the combination treatment of saxagliptin and SCHE as compared to the saxagliptin only group. These results cumulatively indicate enhanced insulin sensitivity in the treatment groups fed with high-fat diet and STZ in comparison to disease control animals. Insulin sensitivity as calculated from HOMA-IR was significantly increased in the disease control group in comparison to the normal control group with $p<0.001$; insulin sensitivity was restored significantly by all the treatment groups: SCHE, saxagliptin, and combination, with $p<0.001$. The results are depicted in Figure 7.

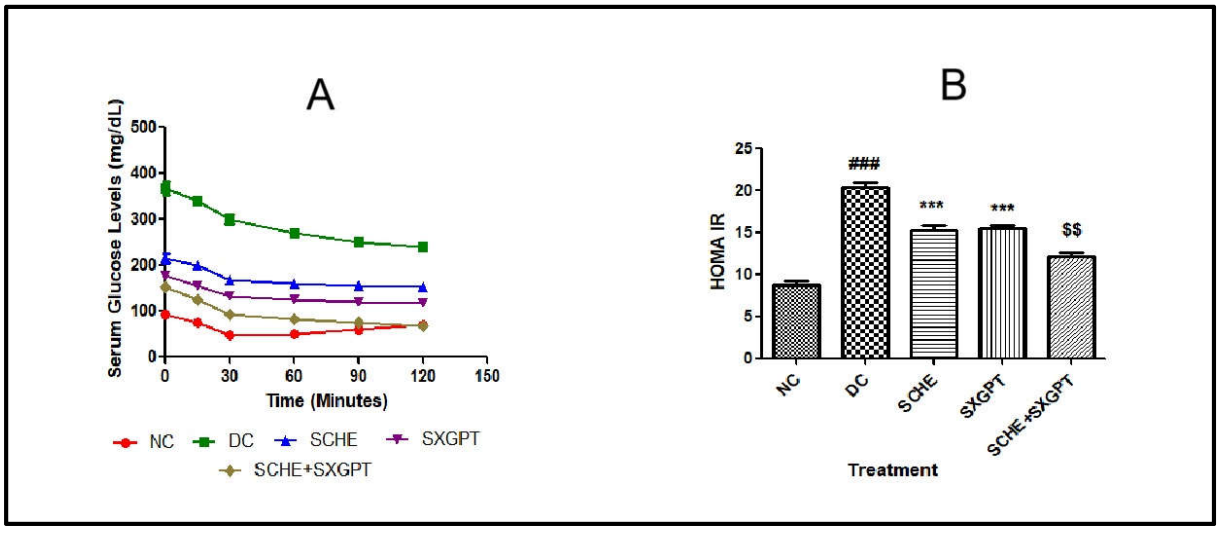

Figure 7. The effect of SCHE, saxagliptin, and combination treatments on insulin tolerance test and HOMA-IR in type 2 diabetic animals. The experimental animals were fed with a 60% high-fat diet for 28 days, and streptozocin (35 mg/kg) was administered on day 14 to induce type 2 diabetes. Experimental animals were subjected to their respective treatments of vehicle, SCHE (200 mg/kg), saxagliptin (1 mg/kg), and co-administration. On day 24, all the experimental animals were administered intraperitoneally insulin at 0.75 IU/kg after overnight fasting. Serum glucose levels were measured at 0, 0.25, 0.5, 1, 1.5, and 2h post-insulin administration. Serum glucose versus time graph was plotted. HOMA-IR was calculated from fasting blood

glucose and insulin level measurements on day 29. Data was represented as mean ± S.D, analysed by one-way ANOVA followed by Bonferroni post hoc test, and $p < 0.05$ was considered to be significant. ###$p < 0.001$ when compared to NC; ***$p < 0.001$when compared to DC and $^{\$\$\$}p<0.001$ when compared to SXGPT.

3.6.4. Effect of treatments on biochemical parameters

There was a significant decrease in serum insulin levels and a significant increase in triglyceride, total cholesterol, and HDL cholesterol levels in the disease control group as compared to the normal control group ($p<0.001$). There was a significant increase in serum insulin levels in all the treatment groups, including SCHE, saxagliptin, and combination treatment groups, with $p<0.001$. The treatments have caused a significant reduction in the triglyceride, total cholesterol, and LDL cholesterol levels as compared to the disease control animals with $p<0.001$. Combination treatment of the SCHE with saxagliptin has caused a significant reduction in triglyceride ($p<0.01$), total cholesterol ($p<0.05$), and LDL cholesterol ($p<0.01$) as compared to the saxagliptin only treatment. However, there was no significant difference observed in serum insulin levels between these two groups. The results of the effect of treatments on serum biochemical parameters are depicted in Figure number 8.

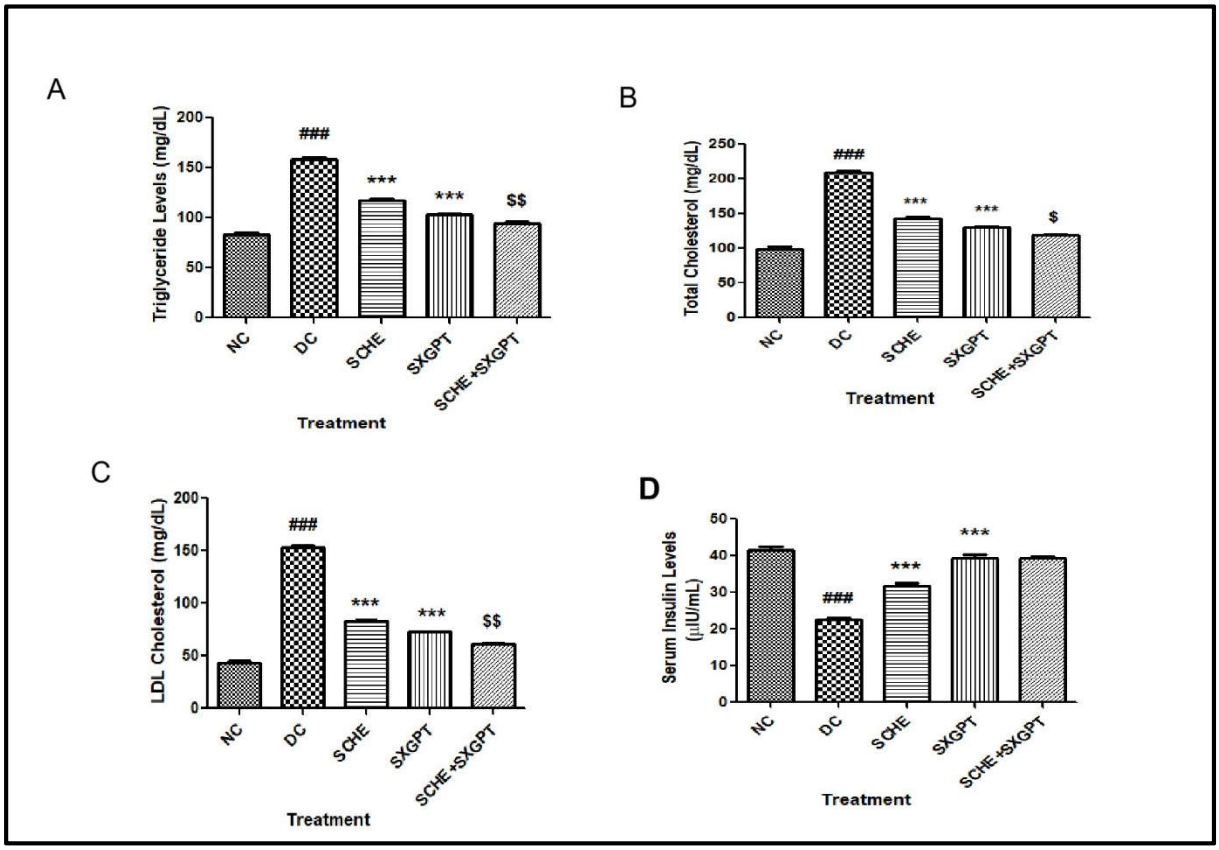

Figure 8. The effect of SCHE, saxagliptin, and combination treatments on serum triglyceride (A), total cholesterol (B), LDL cholesterol (C) and insulin (D) levels in type 2 diabetic animals.

The experimental animals were fed with a 60% high-fat diet for 28 days, and streptozocin (35 mg/kg) was administered on day 14 to induce type 2 diabetes. Experimental animals were subjected to their respective treatments of vehicle, SCHE (200 mg/kg), saxagliptin (1 mg/kg), and co-administration. On day 29, blood was collected after overnight fasting, and serum triglyceride, total cholesterol, LDL cholesterol, and insulin level were measured. Data was represented as mean ± S.D, analysed by one-way ANOVA followed by Bonferroni post hoc test, and $p < 0.05$ was considered to be significant. ###$p < 0.001$ when compared to NC; ***$p < 0.001$ when compared to DC and $^{\$\$}p<0.01$, $^{\$}p<0.05$ when compared to SXGPT.

4. Discussion

In recent years, there has been a growing inclination toward the use of traditionally important medicinal plants, particularly in the management of chronic diseases such as diabetes mellitus. This trend is largely driven by a desire for holistic and natural therapeutic approaches, and due to limitations associated with the adverse effects of the drugs[17]. The concomitant use of traditional system medicines alongside allopathic drugs is a significant contributor to potential drug interactions, particularly because such medicines are often consumed without professional medical consultation[18]. Traditional remedies are widely perceived as inherently safe, leading to their unsupervised use without prescription. This practice of co-administration of herbal or traditional formulations with conventional antidiabetic medications increases the risk of herb-drug interactions[19]. This study aimed to evaluate the herb-drug interaction of the antidiabetic DPP-4 inhibitor, saxagliptin with *Salacia chinensis*, a widely used antidiabetic agent in Ayurveda.

Preliminary *in vitro* studies indicated the inhibitory potential of the SCHE extract on α-glucosidase, α-amylase enzymes, with IC50 238.4 µg/ml and 291.52 µg/ml, respectively. However, it demonstrated poor DPP-4 inhibition with IC50 of 527.39 µg/ml. Insulin resistance is the major predisposing factor for the development of type 2 diabetes. This causes a reduction in glucose uptake into the glucose-sensitive tissues skeletal muscles, adipose tissue and liver[20]. Insulin enhances glucose uptake into adipose tissue and stimulates adipogenesis in the fat tissue. SCHE treatment demonstrated dose-dependent enhancement of glucose uptake into the 3T3-L1 adipocytes in the presence of insulin as compared to the undifferentiated pre-adipocytes and differentiated adipocytes, indicating insulin insulin-sensitizing potential of the extract treatment. Treatment of the 3T3-L1 preadipocyte cells with SCHE has not demonstrated any cytotoxicity in the concentration range of 6.25 to 100 µg/ml.

The dose determination and preliminary SCHE and saxagliptin interaction studies were performed in normal animals. SCHE administration for 3 days consecutively caused dose-dependent reduction in the blood glucose levels in normal animals at 100 and 200 mg/kg doses, from which further interaction studies were performed at 200 mg/kg dose. Administration of saxagliptin has caused significantly higher reduction in blood glucose levels in normal animals as compared to SCHE treatments. Co-administration of the SCHE with saxagliptin caused

significantly higher reduction in animals when compared to the saxagliptin alone administration, indicating possible drug-herb interaction.

Herb-drug interactions are one of the predominant factors responsible for adverse effects. The herb-drug interactions may arise due to pharmacokinetic or pharmacodynamic interactions. Pharmacokinetic interactions are associated with modulations in the absorption, distribution, metabolism and excretion characteristics of the drug by the herb, which causes changes in the plasma concentrations of the drug, thus causing altered therapeutic effect. Whereas, pharmacodynamic interactions are the interactions where there will be an altered pharmacodynamic effect of the drug without any modifications in the plasma level of the drug. This pharmacodynamic interaction arises due to interaction at the receptor level or physiological level[21]. The pharmacodynamic interaction in this study was further evaluated in diabetic animals. Type 2 diabetes was induced in experimental animals by feeding them with a 60% high-fat diet for 28 days, with administration of STZ at 35 mg/kg intraperitoneally on day 14. This caused induction of diabetes mellitus with significantly higher hyperglycaemia and glycated haemoglobin levels as compared to normal animals fed with a normal chow diet. Treatment with SCHE, saxagliptin, and combination treatments significantly reduced the blood glucose and glycated haemoglobin levels, depicting their antidiabetic potential. Combination treatment has produced significantly higher reduction in blood glucose and glycated haemoglobin levels as compared to the saxagliptin only group, indicating possible pharmacodynamic herb-drug interaction. Type 2 diabetes has caused a reduction in the insulin levels, an increase in triglyceride, total cholesterol, and LDL cholesterol in comparison with the normal control animals, which were ameliorated with the treatment groups. The combination treatment has caused a significant reduction in the triglyceride, total cholesterol, and LDL cholesterol, and a non-significant increase in the insulin levels as compared to the saxagliptin only group.

Diabetes mellitus is characterized by reduced glucose and insulin sensitivity of the tissues, which causes reduced utilization of the blood glucose levels by the cells, leading to the development of hyperglycemia, which further causes diabetic associated adverse effects via the formation of glycated end products[22]. The oral glucose tolerance test is used for evaluating the sensitivity of the glucose in physiological systems. Induction of diabetes in disease control animals has caused significantly higher area under curve for the glucose after OGTT in comparison to the normal control animals. Treatments have caused a significant improvement in the area under curve, indicating enhanced glucose sensitivity in the respective groups.

Clinically insulin sensitivity is determined from the insulin tolerance test, which is a direct indicator and HOMA-IR, which is an indirect indicator of insulin sensitivity. Diabetic disease control animals demonstrated reduced decline in the glucose levels after administration of the insulin, whereas normal control animals demonstrated a sharp decline in the blood glucose levels, indicating reduced insulin sensitivity in disease control animals. Similarly, disease control animals demonstrated significantly higher HOMA-IR as compared to normal control animals, indicating reduced insulin sensitivity. Treatments with SCHE, saxagliptin, and their combination have significantly improved the insulin sensitivity in both the insulin tolerance test and HOMA-IR indicating their effect on insulin resistance. The saxagliptin and SCHE combination treatment has demonstrated higher insulin sensitivity as compared to the saxagliptin only group, which might be due to pharmacodynamic interaction between them.

5. Conclusion

In conclusion, the current study results indicate the antidiabetic potential of the SCHE treatment, and there were significant improvements in the serum glucose, glycated haemoglobin, insulin, triglyceride, cholesterol, glucose sensitivity and insulin sensitivity in the combination administration of the SCHE with saxagliptin when compared to the saxagliptin alone group, which might be arising due to the possible pharmacodynamic interaction between saxagliptin and SCHE.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Time (h)	Serum Glucose levels (mg/dL)			
	Saxagliptin (1 mg/kg)	**SCHE (100mg/kg)**	**SCHE (200mg/kg)**	**Saxagliptin + SCHE (200mg/kg)**
0	90.15±3.45	105.30±2.14	98.10±2.46	100.15±4.26
1	82.31±2.38	102.15±2.09	93.05±1.90	91.40±0.95
2	70.75±3.16	97.25±1.78	84.59±1.38	78.20±1.25
3	**56.29±1.64**	**85.43±3.52**	**67.49±1.53**	**54.95±2.10**
4	58.41±2.11	86.59±0.87	68.19±2.09	55.86±1.31
6	61.94±1.43	94.01±2.21	72.48±0.99	57.41±2.01
8	64.20±3.05	103.19±2.05	81.97±1.41	59.48±1.58
10	68.04±1.73	103.80±1.57	95.43±2.03	62.55±1.72
12	74.26±3.10	104.25±2.48	97.78±1.58	66.98±1.59