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

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Abstract:

This study investigated the potential pharmacokinetic 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 the CYP3A4/5 inhibitory potential of *Salacia chinensis* hydroethanolic extract (SCHE) with an IC50 of 31.62 ± 1.39 µg/ml. Incubation of the extract with CYP3A4/5 enzymes reduced the metabolism of saxagliptin by 20% compared to the metabolism of saxagliptin without SCHE incubation. For pharmacokinetic interaction studies in normal animals, they were dosed at 200 mg/kg for 3 days with SCHE followed by saxagliptin, leading to a significant increase in the plasma concentrations of saxagliptin as compared to saxagliptin alone treated group animals. There was also significant increase in Cmax, AUC and MRT observed in combination group as compared to saxagliptin alone group. The pharmacokinetic interaction study in diabetic animals was performed after feeding them with high-fat diet (60%) for 28-days with low dose streptozocin of 35 mg/kg administered on day 14. The animals were treated with combination of SCHE and saxagliptin or saxagliptin alone for 28 days. There were significantly higher concentrations of saxagliptin observed in combination group as compared to the saxagliptin alone group. There was a significant increase in the

pharmacokinetic parameters Cmax, AUC, T1/2 and MRT observed with significant reduction in clearance of the saxagliptin observed in combination group observed as compared to the saxagliptin alone group. Combination of saxagliptin and SCHE demonstrated significantly higher plasma levels in both normal and diabetic animals with significant variation of the pharmacokinetic parameters in diabetic animals observed as the combination was administered for 28 days. This pharmacokinetic interaction of SCHE with saxagliptin might be arising due to CYP3A4/5 inhibitory potential of the SCHE, which reduces the metabolism of saxagliptin. In conclusion, the current study indicates dose adjustment and plasma drug level monitoring of the saxagliptin upon administration with SCHE.

Keywords:

Pharmacokinetic interaction, Drug-Herb interaction, CYP activity, Diabetes mellitus, *Salacia chinensis*, saxagliptin,

1.0. Introduction

Approximately 71% of fatalities worldwide are attributed to non-communicable diseases (NCDs), according to World Health Organization (WHO) reports. The most common condition that contributes to a greater number of NCD-related deaths is diabetes mellitus (DM). Because of its rising incidence and impact on public health and quality of life globally, diabetes mellitus is regarded as an epidemic (Yang and Li, 2023; Forouhi and Wareham, 2022). It is a rapidly rising chronic metabolic disease characterized by high blood glucose levels brought on by either decreased insulin secretion from damage to the beta cells in the pancreas (Type I diabetes mellitus) or insulin resistance that primarily affects insulin-sensitive tissues such as the liver, adipose tissue, and skeletal muscles (Type II diabetes mellitus). It has a major impact on the affected person's health and quality of life (American Diabetes Association, 2014). In 1990, there were approximately 200 million people with diabetes globally; by 2022, that number had increased to around 830 million. Diabetes is associated with microvascular and macrovascular complications, causing kidney failure, blindness, limb amputations, stroke, and heart failure. Diabetes-related hyperglycemia was the direct cause of 1.6 million fatalities worldwide in 2021, accounting for 11% of cardiovascular mortality and almost 2 million deaths from renal impairment (Forouhi and Wareham, 2022).

Diabetes is classified as a lifestyle disorder that can be prevented and treated by changing lifestyle choices, such as choosing a healthy diet with fewer calories, increasing physical

activity by engaging in at least 150 hours of exercise per week, and abstaining from risk factors like alcohol and tobacco use. Insulin analogues and oral antidiabetic medications, 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, are all part of the pharmacological management of diabetes (Elkhalifa *et al.*, 2024; Gieroba *et al.*, 2025). The first-line medication among antidiabetic drugs, either by itself or in conjunction with other medications, is metformin. It is well-tolerated due to its pleiotropic mechanism of action. Dipeptidyl peptidase (DPP)-4 inhibitors serve as incretin-based treatments that mimic the physiological effects of incretins. By inhibiting this enzyme, DPP-4 inhibitors decrease the degradation of incretins such as GIP and GLP-1, which leads to lower glucagon release and increased insulin production from pancreatic beta cells. Because of their relatively mild side effect profile, these medications are now regarded as second-line options for treating T2D in patients who do not respond to metformin. The drugs approved in this category consist of alogliptin, linagliptin, saxagliptin, sitagliptin, and vildagliptin. (Deacon, 2020). Saxagliptin is the most commonly used DPP-4 inhibitor because it dissociates from the enzyme more slowly than other drugs, resulting in l onger-lasting inhibition and better control of serum glucose levels (Dhillon, 2015).

The use of herbal products from traditional medical systems has increased due to the chronic nature of diabetes mellitus and the adverse effect profile of antidiabetic agents. The World Health Organization currently has a list of approximately 400 medicinal plants with antidiabetic properties. These plants are considered safe, with limited adverse effects, and are rich in phytoconstituents that act via multiple pathways. Due to the beneficial effects of medicinal plants, physicians and patients are increasingly inclined towards treatments such as Ayurveda, leading to the use of these herbal products for the treatment of chronic illnesses, including diabetes mellitus (Rombolà *et al.*, 2020).

Ayurveda is the traditional system of medicine in India. Salacia chinensis is a prominent herbal compound used for the treatment of metabolic diseases and inflammatory disorders. Phytochemical studies have reported the presence of various phytoconstituents, including mangiferin, salacinol, salaprinol, quercetin, gallic acid, and kaempferol, among others. Pharmacology studies reported a wide range of pharmacological activities, including antidiabetic potential. The increased use of medicinal plants from traditional systems of medicine by diabetic patients may cause drug-herb interactions of antidiabetic drugs with the medicinal plant, which can arise from the modulation of absorption, distribution, metabolism,

and excretion characteristics of the drugs. Pharmacokinetic herb-drug interactions are prominent in causing adverse effects or a lack of efficacy of the drug, as the phytoconstituents present in plants may impact drug transporters, such as p-glycoprotein, or modulate metabolism by affecting CYP microsomal enzymes, and might affect the excretion of the drug or its metabolite (Peng Y., et al., 2021). In this scenario, the current study aimed to evaluate the pharmacokinetic interaction of the DPP-4 inhibitor saxagliptin with the widely used antidiabetic Ayurvedic drug *Salacia chinensis* in normal and type 2 diabetic rats.

2.0. Materials and Methods:

2.1. Materials

Saxagliptin was provided by MSN Pharmaceuticals (Hyderabad, India) as a gift sample. The solvents used in the study, including ethanol, methanol, hydrochloric acid, and acetonitrile, were procured from Merck (St. Louis, Missouri, USA) and were of analytical grade. The human liver microsomes, CYP isoforms CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP3A4/5, and CYP2C19 were procured from Sigma-Aldrich (Missouri, USA). The CYP substrates phenacetin, bupropion, amodiaquine, diclofenac, dextromethorphan, testosterone, midazolam, and S-mephenytoin, and internal standard telmisartan were procured from Sigma-Aldrich (Missouri, USA). Other chemicals, streptozotocin, disodium hydrogen phosphate, sodium dihydrogen phosphate, potassium tartarate tetrahydrate, sodium hydroxide, 3,5-dinitrosalicylic acid, and DMSO were procured from Sigma-Aldrich (Missouri, USA). High-fat diet (60%) was obtained from the National Institute of Nutrition (Hyderabad, India).

2.2. Preparation of the extract & Phytochemical Analysis

The whole plant of *Salacia chinensis* was dried under shade, finely ground, and sieved. Powdered plant material (100 gm) was added to 70% ethanol (250 ml) in the conical flask and extracted with maceration process using a wrist shaker for 12 h. Further, the flask was allowed to stand for 8 h, and filtered using a 0.22 µm Whatman filter paper. Fresh solvent was added to the menstruum for further extraction, and this process was continued until the solvent had become colorless. The filtrate was subjected to removal of the solvent under reduced pressure utilizing a rotary evaporator (Buchi, Mumbai, India) to prevent degradation of thermolabile constituents in the extract. The flow properties of the extract were enhanced by further removal of the moisture using a freeze dryer, and the extraction efficiency of the yielded extracts was determined using below formula

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

The stock solution (100 mg/ml) of *Salacia chinensis* whole plant hydroethanolic extract (SCHE) was prepared in DMSO by vortexing it for 15 minutes on a vortex mixer. This was further subjected to centrifugation for 10 minutes at 8000 rpm to remove the insoluble extract from the stock solution, and the supernatant obtained was filtered through a 0.22 µm membrane filter. Further dilutions were prepared in the phosphate buffer to prepare working solutions. This extract was subjected to qualitative phytochemical analysis using LC-MS/MS (Lingesh *et al.*, 2019).

2.3. CYP Interaction Study of SCHE

The *in vitro* CYP inhibition assay, performed using human liver microsomes, was designed to evaluate the potential of SCHE to inhibit cytochrome P450 enzyme activity. In this assay, human liver microsomes were incubated with specific CYP probe substrates, as mentioned in Table No. 1, and SCHE extract at concentrations ranging from 0.5 to 500 µg in phosphate buffer (100 mM, pH 7.4). The mixture was pre-incubated at 37°C for about 5 minutes to allow interaction between SCHE and the enzyme. The enzymatic reaction was initiated by adding NADPH (final concentration of 1 mM), which is essential for CYP enzyme activity. The incubation periods for the enzymes were as per Table 1, after which the reaction was terminated by adding a cold organic solvent, acetonitrile. After protein precipitation with acetonitrile, the sample was subjected to centrifugation, and the supernatant was analyzed using LC-MS/MS to quantify the specific metabolites formed by the CYP enzyme. Telmisartan was used as an internal standard for quantifying the metabolites. By comparing the rate of metabolite formation in the presence and absence of the test compound, the percentage enzyme inhibition was determined. From the percentage enzyme inhibition, IC_{50} values were determined for each isoenzyme (Zhang *et al.*, 2019).

S. No.	CYP Isoform	Substrate (Concentration)	Incubation time (minutes)
1	CYP1A2	Phenacetin (35µM)	10
2	CYP2B6	Bupropion (50µM)	10
3	CYP2C8	Amodiaquine (2µM)	10
4	CYP2C9	Diclofenac (10µM)	10

5	CYP2D6	Dextromethorphan (5µM)	10
6	CYP3A4/5	Testosterone (50µM)	10
7	CYP3A4/5	Midazolam (2.5µM)	5
8	CYP2C19	*S*-Mephenytoin (30µM)	20

Table No.1: Details of CYP inhibition assay. Specific substrates were incubated with their respective CYP isoforms in the presence of SCHE (0.5-500 µg) for the duration mentioned above. The enzymatic reaction was terminated, and the concentration of metabolites were measured to determine the percentage inhibition by the SCHE

Saxagliptin is metabolized by the CYP3A4 and CYP3A5 enzymes. Further, an experiment was performed to evaluate the effect of SCHE extract on the metabolism of Saxagliptin using CYP3A4/5 enzyme with human liver microsomes. Saxagliptin was incubated with CYP3A4/5 microsomes in the presence and absence of SCHE extract (100 µg), and the reaction was stopped at 0.25, 0.5, 0.75, and 1h after initiation of the reaction and the concentration of Saxagliptin was determined in the reaction mixture to determine the percentage inhibition of saxagliptin metabolism at different time intervals.

2.4. 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.

2.5. Pharmacokinetic Interaction Study in Normal Animals

The experimental animals were divided into two groups, each containing six animals. The animals were administered treatments as per their group. The plasma concentrations of saxagliptin were measured at 0, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 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 on day 3

- Group II –SCHE combination group: Animals were orally administered with 200 mg/kg of SCHE in 0.1% CMC for 3 days, and on day 3, administered with 1 mg/kg of saxagliptin with a time interval of 1 hour

2.6. Pharmacokinetic 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 (35 mg/kg) streptozotocin (STZ) intraperitoneally. The same experimental animals were utilized for both pharmacodynamic and pharmacokinetic interaction studies in diabetic animals. For pharmacodynamic interaction studies, the groups included a normal control, which was fed normal chow, and a disease control, SCHE, Saxagliptin, and a combination group. All groups were fed a high-fat diet (60%) for 28 days, with streptozotocin injection administered on day 14. As the pharmacokinetic interaction study aims to assess the impact of SCHE on the plasma concentrations of saxagliptin in diabetic animals, a pharmacokinetic interaction study was conducted, and the plasma levels of saxagliptin were measured in both the saxagliptin group and the combination of SCHE and saxagliptin group. The animals were intraperitoneally injected with 35 mg/kg of streptozotocin on day 14 for the induction of type 2 diabetes (K *et al.2005*). The experimental design consisted of the following groups.

- Group I – 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 II - Co-administration group: Animals were fed with a high-fat diet (60%) and orally administered with saxagliptin at 1 mg/kg followed by 200 mg/kg SCHE extract for 28 days, and a low dose (30 mg/kg) STZ on day 14

The pharmacokinetic interaction study was performed on day 28, after administration of the last dose of treatment. Blood samples were collected from these two experimental group animals at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h post administration of the last dose. Plasma samples were prepared from these blood samples by centrifugation at 5000 rpm for 5 minutes at 4ºC, and the samples were kept at -20ºC for further estimation of the saxagliptin concentrations using LC-MS/MS

2.7. Chromatographic Method

Serum concentrations of saxagliptin in the experiments were determined using LC-MS/MS (Waters, Japan). The system was equipped with a variable wavelength programmable UV or photodiode array detector. A reverse-phase HPLC system with a C8 column of 5 µm particle size; 100 mm length x 4.6 mm diameter was utilized as the stationary phase. The mobile phase for this study consisted of a 60:40 mixture of phosphate buffer (aqueous phase) and acetonitrile (organic phase), which was used with an isocratic method. The mobile phase flow rate was 1.2 mL/min, and the effluent was monitored at a wavelength of 229 nm. The internal standard for this HPLC method was metformin, and the serum levels of saxagliptin were determined from the ratio of saxagliptin peak area and internal standard peak area. Empower software was used for the analysis and interpretation of data (Shantikumar *et al.*).

2.8. Sample Preparation & Pharmacokinetic Analysis

The serum sample (100µl) was taken in a micro centrifuge tube, mixed with internal standard (100µl), and mixed well. To this sample mixture, 200 µL of acetonitrile was added to precipitate the protein. The resultant mixture was vortexed and centrifuged at 3000 rpm for 5 minutes. The collected supernatant was filtered through a 0.45 µm membrane filter, and the resultant filtrate (20µl) was injected into LC-MS for analysis of saxagliptin. The concentrations of saxagliptin in plasma samples from normal and diabetic animals at various time intervals were determined using the calibration curve. The concentration versus time graph was plotted, and the concentration-time data was utilized for determining the following pharmacokinetic parameters: maximum concentration (C_{max}), time to reach maximum concentration (T_{max}), elimination rate constant (K_e), elimination half-life ($t_{1/2}$), area under curve (AUC), clearance (CL) utilizing Phoenix WinNonlin software for all the groups. The pharmacokinetic parameters from the saxagliptin group were compared with those of the saxagliptin and SCHE co-administration group in both normal and diabetic animals (Surendran *et al.*).

2.9. Statistical Analysis

The data in this study are expressed as Mean ± SD, and the results were analyzed using one-way analysis of variance 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 to remove residual solvent. The obtained extract was dissolved in DMSO (100 mg/mL), vortexed for 5 minutes, and then centrifuged at room temperature for 5 minutes at 8000 rpm. The resulting supernatant was used as the stock solution for in vitro studies. The extract was suspended in 1% sodium carboxymethyl cellulose (100 mg/ml) and utilized for pharmacokinetic interaction studies in normal and diabetic animals.

3.2. Qualitative Phytochemical Analysis of SCHE

The qualitative phytochemical analysis of SCHE using LC-MS/MS and NIST11 library indicated the presence of phytoconstituents such as mangiferin, salacinol, kaempferol, lupeol, syringic acid, balarenone, acetyl barlerin, barlerinoside, etc. The details of the phytoconstituents are presented in Table 2.

Sr no.	**Phytoconstituents**	**Pub chem. ID**	**Rt**	**Mol. Wt.**	**Pub chem. Wt.**	**m/z**
1.	Syringic acid	10742	17.26	199	198	M+H
2.	Mangiferin	5281647	32.34	422	422.3	M-3H
3.	Kaempferol	5280863	32.24	283	286	M-3H
4.	Lupeol	259846	23.62	425	426	M-H
5.	Salacinol	6451151	6.96	334	334.4	M+2H
6.	Balarenone	16724214	26.87	466	462	M+4H

Table 2. Details of phytoconstituents identified by LC-MS/MS analysis of Salacia chinensis whole plant hydro-ethanolic extract.

3.3. Effect of SCHE Extract on CYP Inhibition

Incubation of the SCHE with CYP enzymes demonstrated intermediate inhibitory activity on CYP3A4/4 enzyme with an IC50 of 31.62±1.39 µg/ml. It demonstrated weak inhibitory activity

against CYP1A2, CYP2B6, and CYP2C8, with IC50 values of 92.53 ± 4.20, 80.29 ± 3.92, and 125.31 ± 5.28 µg/mL, respectively. Whereas SCHE demonstrated no inhibitory activity on CYP2C9, CYP2D6, and CYP2C19, with IC50 values higher than 500 µg/mL. The IC50 values of SCHE for CYP3A4 enzymes are depicted in Figure 1.

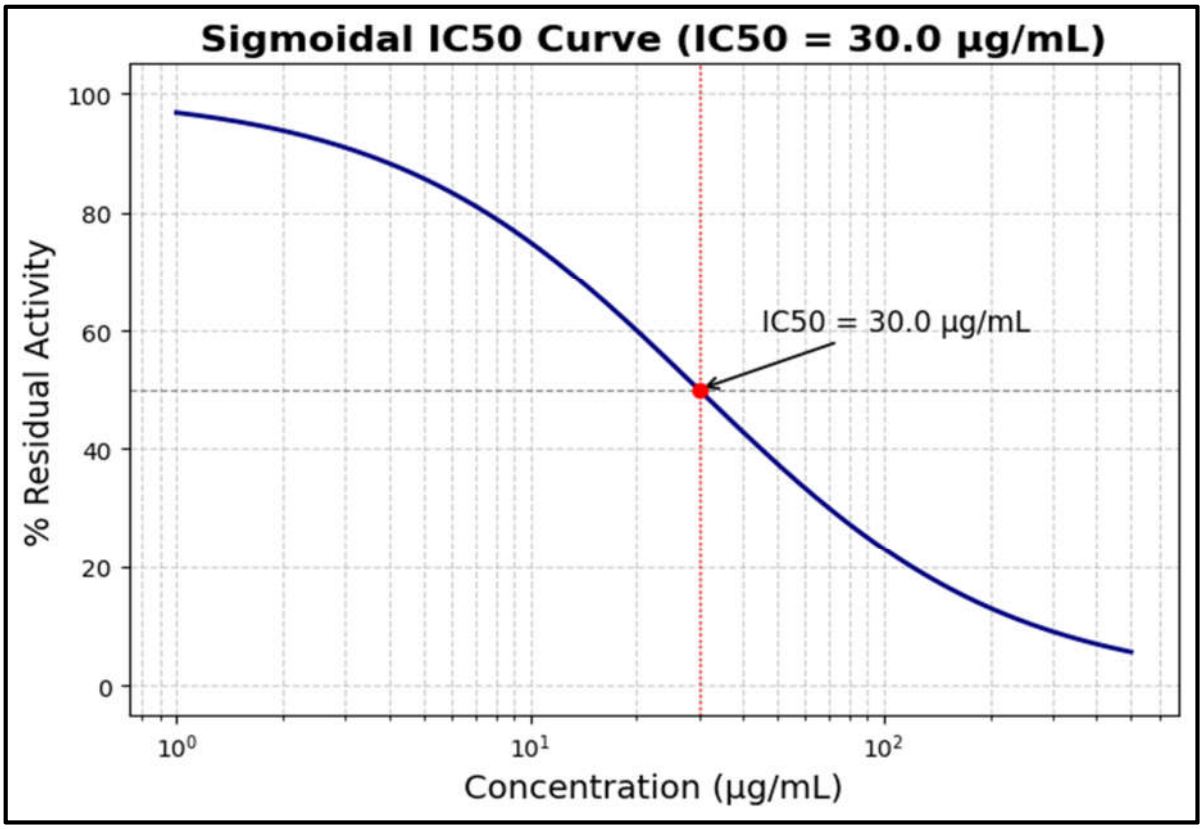

Figure 1. Effect of SCHE on CYPA4/5 Inhibition.

For studying the effect of SCHE extract on the metabolism of saxagliptin *in vitro*, human liver microsomes were used. Saxagliptin was incubated with liver microsomes in the presence and absence of the SCHE extract (100 µg/ml), and the reaction was stopped at 0.25, 0.5, 0.75, and 1 hour after initiation of the reaction, and the concentration of Saxagliptin in the reaction mixture was measured. These studies indicated significant metabolic inhibition of saxagliptin in the presence of the SCHE extract, with 20% higher levels of saxagliptin in liver microsomes in the presence of the extract compared to saxagliptin alone at the end of a 1-hour incubation.

3.4. Pharmacokinetic Interaction Study in Normal Animals

To evaluate the effect of SCHE treatment on the pharmacokinetics of saxagliptin in normal

animals, the extract was administered to the animals for 3 days at a dose of 200 mg/kg. On day 3, saxagliptin was administered in combination, and the plasma concentrations and pharmacokinetic parameters were compared with those of saxagliptin administered alone. Plasma concentrations of saxagliptin were measured using LC-MS/MS; the total ion chromatogram (TIC) of saxagliptin is depicted in Figure 2. Saxagliptin eluted with a retention time of 2.90 in this specific mobile phase. The developed method demonstrated linearity in the concentration range of 2 to 200 ng/mL, with an R^2 of 0.9913, as shown in Figure 3.

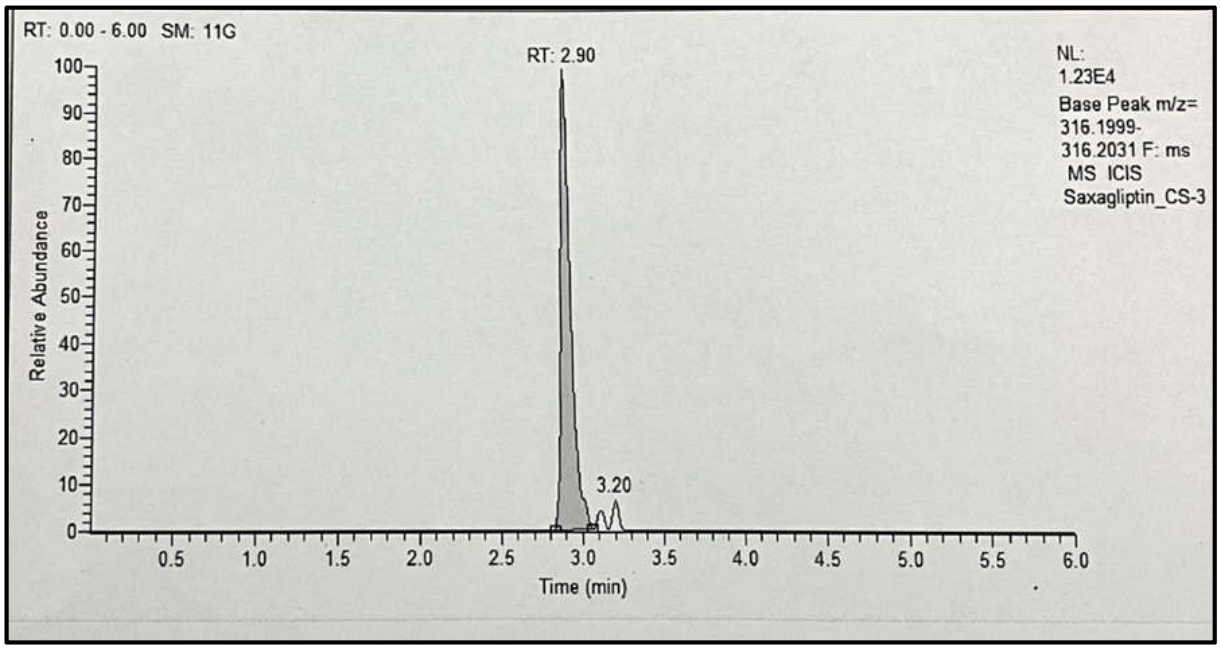

Figure 2. Total Ion Chromatogram of Saxagliptin.

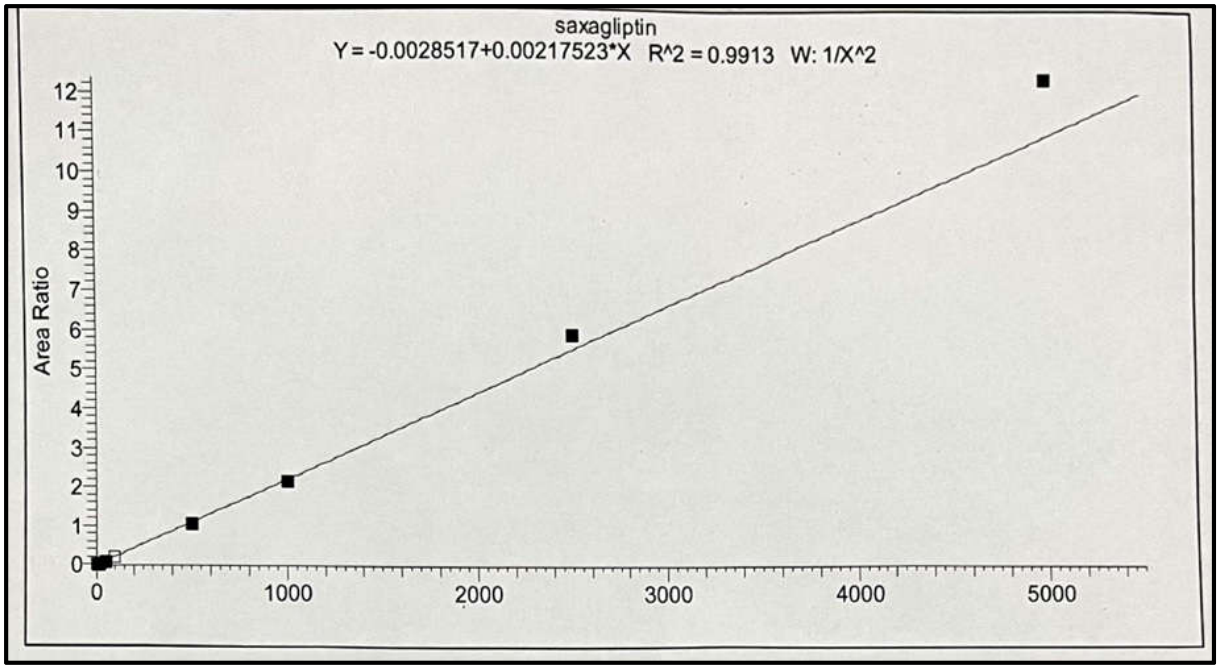

Figure 2. Calibration curve of Saxagliptin.

The plasma concentration-time data for the saxagliptin alone group and the combination group are demonstrated in Figure 4. These results indicated higher concentrations of saxagliptin in the combination group compared to the saxagliptin-alone group, suggesting a pharmacokinetic interaction between SCHE and saxagliptin upon co-administration. Furthermore, pharmacokinetic parameters were calculated from the plasma concentration-time data of saxagliptin for both groups. These pharmacokinetic parameter data indicated significantly ($p<0.001$) higher Cmax in the combination group (123.80±15.26) as compared to the saxagliptin alone group (123.80±15.26). There was a significant increase in AUC observed in the combination group as compared to the saxagliptin alone group, and there were no significant changes observed in other pharmacokinetic parameters. The pharmacokinetic parameter data for both groups are depicted in Table 3.

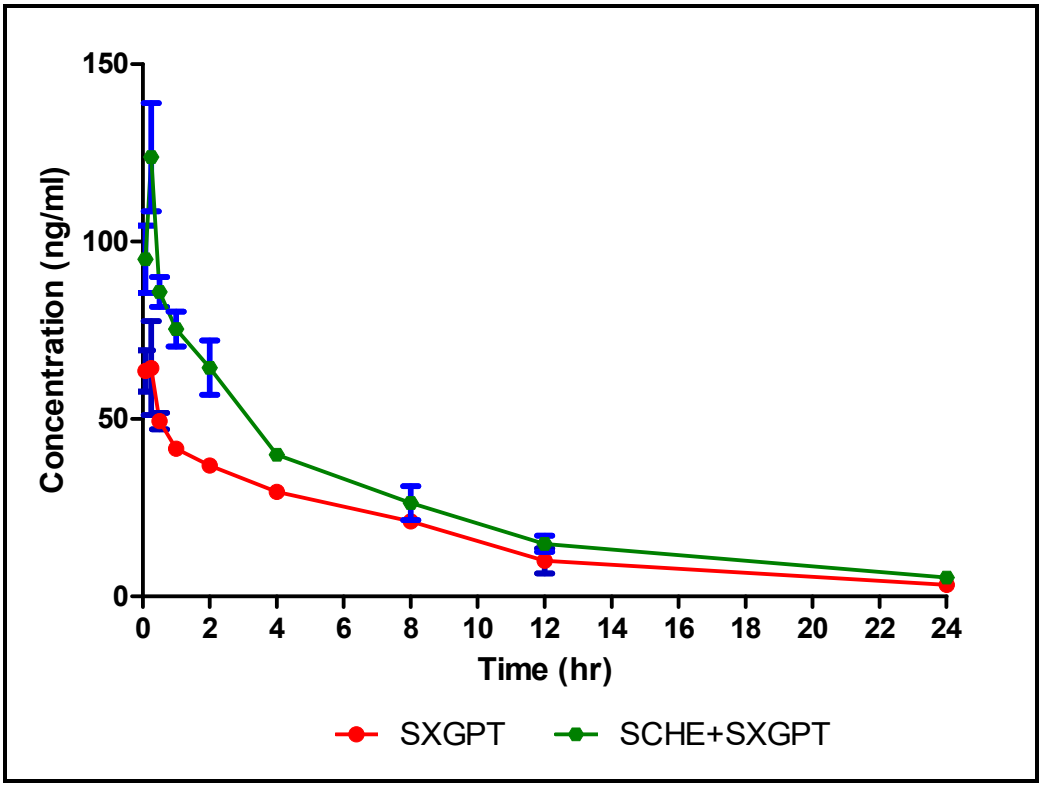

Figure 4. The effect of SCHE treatments on serum saxagliptin levels and interaction with saxagliptin in normal animals. The experimental animals were divided into two groups, each consisting of 6 animals. The animals were treated for three days with SCHE at 200 mg/kg, followed by saxagliptin at 1 mg/kg on day 3 in the combination group, and the saxagliptin alone group was treated with saxagliptin at 1 mg/kg. On day 3, blood samples were collected at 0, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h time intervals, and serum saxagliptin levels were measured.

PK Parameters	**Saxagliptin**	**Saxagliptin + SCHE (200mg/kg)**
C_{max} (ng/ml)	70.87 ± 7.77	123.80 ± 15.26***
T_{max} (hr)	0.14 ± 0.10	0.25 ± 0.00*
AUC_{last} (ng.hr/ml)	400.69 ± 35.09	537.75 ± 60.57**
AUC_{inf} (ng.hr/ml)	428.99 ± 42.12	567.77 ± 65.56**
$T_{1/2}$ (hr)	5.78 ± 0.07	5.78 ± 0.10
MRT (hr)	7.07 ± 0.61	5.90 ± 0.55*
CL_F (ml/min/kg)	39.11 ± 3.99	29.62 ± 3.47

Table 3. The effect of SCHE and saxagliptin co-administration on pharmacokinetic parameters of saxagliptin in normal animals as compared to saxagliptin alone-treated animals.

3.5. Pharmacokinetic Interaction Study in Diabetic Animals

The effect of SCHE treatment on plasma concentrations and pharmacokinetic parameters of Saxagliptin in diabetic animals was evaluated using a high-fat diet and a low-dose streptozocin model. Unlike the pharmacokinetic interaction study in normal animals, in this study, the animals were treated for 28 days with SCHE and saxagliptin in the combination group and with saxagliptin alone in the saxagliptin-only group.

There was a significant increase in saxagliptin levels observed in the combination group as compared to the saxagliptin-only group, as depicted in Figure 5. The increase in plasma concentrations observed in this study was higher than that reported in the study on normal animals. Furthermore, pharmacokinetic parameters were calculated from the plasma concentration-time data of saxagliptin for both groups. These pharmacokinetic parameter data indicated significantly ($p < 0.001$) higher C_{max}, AUC, and $T_{1/2}$, with a significant reduction in the clearance of saxagliptin in the combination group. These pharmacokinetic parameters are depicted in Table 4.

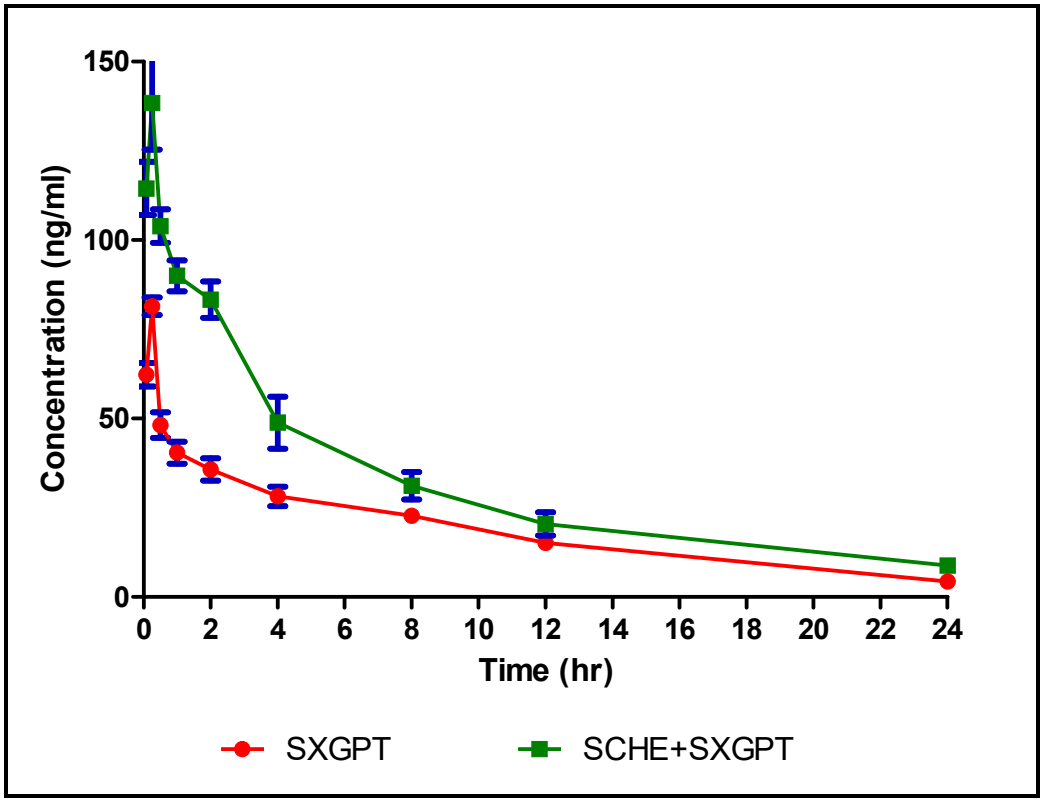

Figure 5. The effect of SCHE treatments on serum saxagliptin levels and interaction with saxagliptin in diabetic animals. The experimental animals were fed with a high-fat diet (60%) for 28 days and intraperitoneally administered with 35 mg/kg on day 14 for the induction of

diabetes. The animals were treated for 28 days with SCHE at 200 mg/kg, followed by saxagliptin at 1 mg/kg in the combination group, and the saxagliptin alone group was treated with saxagliptin at 1 mg/kg. On day 28, blood samples were collected at 0, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h time intervals, and serum saxagliptin levels were measured.

PK Parameter	**Saxagliptin**	**Saxagliptin + SCHE (200mg/kg)**
T_{max} (hr)	0.25±0.00	0.25±0.00
C_{max} (ng/ml)	81.47±2.44	138.44±15.26***
AUC_{last} (ng.hr/ml)	430.22±25.01	744.58±79.43***
AUC_{inf} (ng.hr/ml)	474.35±41.31	863.59±141.26***
$T_{1/2}$ (hr)	6.93±0.02	8.66±0.05**
MRT (hr)	7.70±0.54	7.12±0.52
CL_F (ml/min/kg)	35.32 ± 3.22	19.63 ± 2.99

Table 4. The effect of SCHE and saxagliptin co-administration on the pharmacokinetic parameters of saxagliptin in diabetic animals as compared to saxagliptin alone-treated animals

4. Discussion

In recent years, there has been a growing global interest in the use of traditionally significant medicinal plants, particularly for the management of chronic conditions such as diabetes mellitus. This shift is largely motivated by the pursuit of holistic and natural therapeutic alternatives, as well as by the limitations and adverse effects associated with conventional pharmacological treatments. However, the concurrent use of traditional herbal remedies alongside allopathic medications raises considerable concerns regarding potential drug–herb interactions, especially given that such practices often occur without professional medical supervision (Borse *et al.*, 2019). Ayurvedic drugs with antidiabetic properties are generally co-administered along with antidiabetic medications, which might cause herb-drug interactions. These herbal drugs are enriched with vital secondary metabolites, which are predominantly responsible for their therapeutic effects. Several research findings have indicated the modulatory effects of herbal drugs and their phytoconstituents on the absorption, distribution, metabolism, and excretion characteristics of co-administered drugs, thereby altering the pharmacokinetic parameters of the drug and leading to pharmacokinetic herb-drug interactions (Li *et al.2022*). DPP-4 inhibitors are widely used antidiabetic agents, which are metabolized by CYP3A4 microsomal enzymes into active metabolites (Giorda *et al.*, 2014). In this scenario, the current study aimed to investigate the CYP inhibitory potential of the Ayurvedic drug Salacia chinensis and evaluate the pharmacokinetic interaction of this extract with saxagliptin in normal and diabetic animals.

Phytochemical analysis of the SCHE demonstrated the presence of mangiferin, syringic acid, kaempferol, salacinol, and lupeol. Pharmacokinetic interactions occur due to modulation in the absorption, distribution, metabolism, and excretion properties of the drug, thus leading to modifications in the plasma concentrations of the drug. Among these characteristics, metabolism is the predominant causative factor responsible for pharmacokinetic interactions. CYP 450 enzymes play a predominant role in the metabolism of drugs, which generally causes the inactivation of the drug and also increases its hydrophilicity, thus enhancing its ability to be excreted through the kidneys (Hakkola *et al.*, 2020).

The CYP inhibitory studies of the SCHE with various human CYP microsomes indicated significant inhibitory potential of SCHE on CYP3A4/5 with lower IC50 and non-significant inhibitory potential on other CYP enzymes with higher IC50 values. These results indicate the inhibitory potential of the SCHE extract metabolism of saxagliptin, which is predominantly

metabolized by CYP3A4. This inhibition of the metabolism might cause pleiotropic effects on the pharmacodynamics of the saxagliptin, as the metabolism of saxagliptin produces the active metabolite 5-hydroxy saxagliptin, which also inhibits the DPP-4 enzyme. Research findings indicated the CYP inhibitory potential of mangiferin with effect on CYP2D6, CYP3A4, and CYP3A5, whereas kaempferol demonstrated inhibitory effect on CYP3A4 microsomes in in vitro studies. The CYP3A4 inhibitory potential of SCHE might be due to these phytoconstituents.

Herb–drug interactions represent one of the major contributors to adverse effects associated with concomitant therapy. These interactions can occur through either pharmacokinetic or pharmacodynamic mechanisms. Pharmacokinetic interactions occur when herbal constituents alter the absorption, distribution, metabolism, or excretion of a drug, resulting in changes in plasma drug concentrations and, consequently, alterations in therapeutic efficacy or toxicity. In contrast, pharmacodynamic interactions occur when herbal compounds influence the pharmacological action of a drug without affecting its plasma concentration, thereby altering the overall therapeutic response (Czigle *et al.*, 2023). The pharmacokinetic interaction studies in normal animals indicated a higher Cmax and area under the curve in the combination group compared to the saxagliptin alone group; this may be due to the CYP inhibitory potential of the SCHE extract. Saxagliptin is an oral antidiabetic agent that is absorbed rapidly into systemic circulation. There was no significant difference observed in the time to reach maximum plasma concentration, and the absence of carrier-mediated uptake for saxagliptin absorption denotes a lack of interaction of SCHE with saxagliptin at the absorption level.

The pharmacokinetic interaction study in diabetic animals revealed significantly higher modifications in plasma concentrations and pharmacokinetic parameters of saxagliptin compared to normal control animals. This may not be due to hyperglycemia associated with diabetes, as studies have indicated an inconsistent effect of diabetes on CYP enzymes, which varies between isoforms and has tissue specificity, potentially not causing a significant effect on the metabolism of saxagliptin. However, this difference may arise from differences in the duration of SCHE treatment in normal and diabetic animals. Diabetic animals were administered SCHE in a combination group for 28 days, which may have caused significantly higher CYP3A4/5 inhibition compared to normal animals, where the treatment duration was 3 days. As diabetes is a chronic disease, where antidiabetic medications are administered continuously for prolonged periods, both in the traditional and modern systems of medicine,

this indicates a higher probability of pharmacokinetic interaction if SCHE is co-administered with saxagliptin (Gupta *et al.*, 2017).

In conclusion, the current study results indicate the in vitro CYP3A4/5 potential of SCHE extract and alterations in plasma concentrations and pharmacokinetic parameters of saxagliptin upon co-administration in both normal and diabetic animals. This necessitates cautious therapeutic usage of SCHE with antidiabetic agents due to its pharmacokinetic herb-drug interaction, which might cause toxicity.

Conflict of Interest

The authors declare that they have no conflict of interest

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