PHARMACODYNAMIC AND PHARMACOKINETIC INTERACTIONS OF REPAGLINIDE AND SITAGLIPTIN WITH PIPERINE IN ANIMAL MODELS

Thesis Submitted for the Award of the Degree of

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DECLARATION

I, hereby declare that the work reported in the thesis entitled **"Pharmacodynamic and Pharmacokinetic Interactions of Repaglinide and Sitagliptin with Piperine in Animal Models"** has been carried out by me under the supervision of Dr. Sazal Patyar, (Department of Pharmacology, LPU, Jalandhar, Punjab, India) during the session 2018- 2023. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled **"Pharmacodynamic and Pharmacokinetic Interactions of Repaglinide and Sitagliptin with Piperine in Animal Models"** submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the **Pharmacology Department**, is a research work carried out by P. Sagar, 41800107, which is a bonafide record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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ABSTRACT

Background

Diabetes causes excessive glucose levels due to inadequate insulin production. According to estimates, 72.96 million of India's adult population will get diabetes. Piperine has an anti-diabetic property and diabetic patients are more likely to include piperine in their normal diet. But piperine inhibits the CYP450 enzymes as well as P-gp and has an effect on β-cells which has the ability to release insulin which can show its influence on antidiabetic drugs. With this context in mind, this research aimed to explore piperine's effect on chosen antidiabetic drugs (Repaglinide and Sitagliptin) through Pharmacodynamic/Pharmacokinetic interaction studies in animal models (rat and rabbits).

Objectives

- To investigate the Pharmacodynamic interactions of selected anti-diabetic drugs (Repaglinide & Sitagliptin) with piperine in normal and diabetic animal models.
- To investigate the Pharmacokinetic interactions of selected anti-diabetic drugs (Repaglinide & Sitagliptin) with piperine in normal and diabetic animal models.

Materials and Methods

For the study of pharmacodynamic interactions, normal and diabetic animals were treated with selected anti-diabetic drugs (Repaglinide/Sitagliptin), piperine, and combination of Repaglinide with piperine & Sitagliptin with piperine. Drugs were administered for 21 days and blood samples collected at pre-determined intervals on 1, 3, 7, 14 and 21st day. The GOD-POD and ELISA methods were used on plasma samples to determine glucose and insulin concentrations. While HPLC analysis of plasma samples was done on days 1 & 21 at predetermined time intervals for evaluation of pharmacokinetic parameters. Statistical comparisons of anti-diabetic drugs (Repaglinide and Sitagliptin) alone and in combination with piperine were performed to assess the Pharmacodynamic/Pharmacokinetic interactions.

Results

The results of Repaglinide-Piperine interaction study indicated that Repaglinide in combination with Piperine showed significant antihyperglycemic action in diabetic animals (rats and rabbits) and significant (hypoglycemic impact in normal animals (rats and rabbits) as compared with Repaglinide alone. Assessment of PK parameters showed that piperine enhanced Repaglinide's bioavailability. Similarly, the results showed a significant anti hyperglycemic action in diabetic animals (rats and rabbits) and no hypoglycemic impact in normal animals (rats and rabbits) when Sitagliptin was co-administered with piperine. PK assessment revealed that piperine increased the bioavailability of Sitagliptin.

Conclusion

The findings of this investigation led to the conclusion that Repaglinide and Sitagliptin have Pharmacodynamic/Pharmacokinetic interactions with piperine. Piperine increases the bioavailability of Repaglinide and Sitagliptin. So close monitoring is required and caution should be exercised when this combination is given for therapeutic benefit.

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P. Sagar

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Abbreviations

DM	Diabetes Mellitus
WHO	World Health Organization
PD	Pharmacodynamics
PK	Pharmacokinetics
HDI	Herb Drug Interaction
CYP	Cytochrome P450
cAMP	Cyclic Adenosine Monophosphate
IDF	International Diabetes Federation
SJW	St. John Worts
IGT	Impaired Glucose Tolerance
GLP-1	Glucagon like peptide-1
GIP	Glucose dependent insulinotropic polypeptide
GABA	Gama Aminobutyric Acid
CNS	Central Nervous System
ABC	ATP Binding Cassette
NAD^+	Nicotinamide Adenine Dinucleotide (oxidized)
PARP-1	Poly-ADP-ribose-polymerase-1
ATP	Adenosine Triphosphate
NA	Nicotinamide
STZ	Streptozotocin
SSRI	Selective Serotonin Reuptake Inhibitors

1. INTRODUCTION

"The person who takes medicine must recover twice, once from the disease and once from the medicine." - William Osler (Canadian Physician, 1849-1919) [1].

Medicines are known to cause safety issues, so healthcare professionals should properly monitor their use. Patients generally assume that natural products or herbal remedies are better and safe. So concomitant intake of medicines and herbal/natural treatments is very common. Because these items are often self-administered to treat or prevent the beginning of a medical illness, concurrent use of drugs is possible [2].

It is difficult to get an accurate picture regarding the concomitant use of natural products and conventional drugs, as patients rarely tell their doctors [3]. With the rise in sales of herbal products in India, with an anticipated INR 300 billion in 2018 and INR 710.87 billion predicted by 2024, this can assume that concurrent use of herbal products is widely prevalent in India [4].

According to a survey study from an Indian tertiary care hospital, roughly 58% of doctors and 28% of patients use herbal therapy with conventional medicines. This proportion may even be higher for patients who suffer from gastrointestinal problems, sleeplessness, hepatic disease, chronic pain, melancholy, menopause and asthma [3].

The concomitant use of herbs with drugs raises concerns regarding HDIs, which may be due to any alteration in the "victim" drug's pharmacokinetics (PK) and/or pharmacodynamics (PD) caused by the herb or herbal product. HDIs can affect both PK and PD of co-administered drugs, resulting in drug-related toxicity or reduced effectiveness.

The herb and medication targets have similar receptors or signalling pathways as PD mechanisms. In certain cases, the drug may not considerably regulate an off-target mechanism on its own. Still, concurrently administered herbs might enhance off-target modulation, resulting in an unanticipated adverse event. As a result of these mechanisms, the biological activity of an herbal product opposes, improves, or synergizes that of the victim drug [5]. The most often reported PD-based HDIs to

involve antithrombotic medicines since numerous herbal items contain anticoagulant, antiplatelet, and/or fibrinolytic characteristics. As an example, ginkgo and garlic have been related to a higher risk of bleeding while using warfarin [6, 7]. Other PD interactions that have been frequently documented include drugs acting on the central nervous system. When combined with SSRIs such as paroxetine, sertraline, nefazodone and fluoxetine, St. Johns Worts (SJW) can cause a manic episode or serotonin syndrome. The underlying mechanism most likely contains an additive effect of SJW on serotonin reuptake [5, 6].

As previously stated, PK-based HDIs make up the vast majority of HDIs. Because of inhibition or stimulation of drug-metabolizing enzymes and xenobiotic transporters by the herbal products, these interactions change the victim drug's clearance or systemic exposure [5, 6].

HDIs can be a double-edged sword, posing both dangers (adverse drug effects) and advantages (enhancing performance). Thus, HDIs may enhance or reduce the activity of the drug or result in drug-related toxicity. So, it is very important to explore such putative HDIs to avoid harmful interactions and exploit the beneficial ones for managing chronic illnesses with high morbidity and mortality rates like diabetes mellitus (DM).

DM is a disease in which the body does not produce enough insulin or does not respond to it correctly, resulting in abnormally high blood glucose levels [8]. Although different classes of antidiabetic medications are available on the market, the use of herbal products as supplementary medicines is very prevalent in diabetic patients. Black pepper is extensively used in various ailments, including DM, as per the traditional Indian system of therapy [9]. In India, it is commonly used as a household spice and is taken by diabetic patients as an herbal remedy [10]. One of its active phytoconstituent is piperine, which has exhibited various pharmacological effects like hepatoprotective, anti-inflammatory, antioxidant, anti-ulcer and antidiabetic [11].

Piperine has been reported to interact with various therapeutic agents, altering their absorption, membrane transport or metabolism. The human CYP3A4, CYP1A2,

CYP2D6, and CYP2C9 enzymes, as well as the P-gp transporters, which are both essential for the metabolism and movement of xenobiotics and other metabolites, have all been reported to be inhibited by piperine [12]. Repaglinide and sitagliptin are mainly metabolized by CYP3A4 and CYP2C8 enzymes [13, 14], so their interaction with piperine is very likely. Earlier studies have reported PK/PD interactions of piperine with different antidiabetic drugs. However, there is no report/evidence on the influence of piperine on the PD/PK of repaglinide and sitagliptin. So, this study aimed to investigate the effect of piperine on PD & PK of selected antidiabetic drugs (repaglinide and sitagliptin) in animal models.

2. LITERATURE REVIEW

2.1. Diabetes mellitus (DM)

DM is a disease in which the body does not generate enough insulin or cannot react appropriately to it, ensuing in highly elevated blood glucose levels. Blood glucose levels in healthy people range from 70 to 110 mg/dL. Excessive glucose levels cause DM symptoms and consequences [15].

2.1.1. Epidemiology

As per International Diabetes Federation, in 2010, 285 billion persons (6.4 percent of the worldwide adult population) were diagnosed with DM. By 2030, it is expected to grow to 439 million people [16]. In India, 72.96 million persons are estimated to have been affected by DM. In urban areas, the occurrence ranges from 10.9 to 14.2%. Still, in rural India, the prevalence ranges from 3.0-to 7.8% amongst those aged 20 and over, with a considerably higher majority amongst aging more than 5 decades as per INDIAB Study [17].

2.1.2. Etiological classification of DM

DM can be categorized into different types based on causative factors:

i) Prediabetes

Prediabetes is a disorder that occurs when blood sugar levels are abnormally high but not high enough to be diagnosed as DM. Fasting blood sugar levels in people with prediabetes range from 100-125 mg/dL or 140-199 mg/dL 2 hrs after a glucose tolerance test. The likelihood of getting DM and heart disease rises with prediabetes [15].

ii) Type 1 DM

In type 1 DM, also termed insulin-dependent DM or juvenile-onset DM, the body's immune system injures the insulin-producing pancreatic cells, and over 90% of them are irreversibly destroyed. Thus, the pancreas generates little to no insulin. Only 5-10% of people with DM acquire type-1 illness before 30 [15, 18].

iii) Type II DM

In type-2 DM, also termed non-insulin-dependent DM or adult-onset DM, the pancreas often generates insulin, frequently at more than normal levels, especially in the first stages of the illness. However, since the body becomes resistant to insulin's actions, the amount of insulin required is inadequate. Approximately 26% of persons aged 65 and older have type-2 DM. Insulin resistance may be caused by high dosages of corticosteroids, acromegaly, pregnancy, and pancreatitis [15, 18].

iv) Gestational diabetes

Pregnancy exacerbates pre-existing type-1 and type-2 DM [19]. Obesity, hyperinsulinemia, and insulin resistance in women may progress to gestational DM. Gestational DM affects around 5% of pregnancies and raises the chance of developing type-2 DM later in life. [20]. DM during pregnancy increases the mother's and the fetus's risk of morbidity and mortality. Neonates are susceptible to respiratory distress, hyperviscosity, hyperbilirubinemia, hypoglycemia, and several other conditions [15, 19, 20].

v)Drug-induced DM

Various pharmacological agents (Table 1) affect glucose homeostasis resulting in either hypo- or hyperglycemia [21].

Table 2.1. List of medications that may cause DM [22].

Class of drugs	Drugs
Antihypertensives	β-adrenergic blockers, calcium-channel blockers, α-adrenergic blockers and minoxidil.
Antiretroviral drugs	Protease inhibitors didanosine, pentamidine, lamivudine, and stavudine
Lipid-lowering agents	Niacin
Bronchodilators	Theophylline and β2-adrenergic agonists
Immunosuppressive agents	Tacrolimus and cyclosporin
Antibiotics/antimetabolites	Nalidixic acid, isoniazid, asparginase and rifampin
Psychotropic drugs	Levodopa/dopamine, phenothiazines,

Class of drugs	Drugs
	chlordiazepoxide, morphine, and lithium,
Hormones	Adrenocorticotropin, corticosteroids, octreotide, thyroid hormones, oral contraceptives, high-dose anabolic steroids, and megestrol acetate
Toxins	Vacor (rodenticide), alcohol, cyanide, and streptozocin

vi) Genetic defects of insulin action and β-cell function

Mutations in genes implicated in glucose sensing, function, development, and regulation of beta cells are responsible for Maturity Onset Diabetes of the Young (MODY) [23]. In MODY, insulin secretion is reduced and there is minimal to no insulin resistance [23]. It is split into two categories: neonatal and MODY-like. Neonatal DM develops in the first 6 months of life and may be either temporary or persistent [23, 24].

vii) Pancreatic diseases

Severe damage to the pancreas or pancreatic diseases can cause DM. Pancreatitis, trauma, and cancer are the most prevalent causes. Chronic pancreatitis can cause pancreatic inflammatory/fibrotic alterations, leading to DM. Viscid, thick secretions may induce inflammation, occlusion, and death of tiny pancreatic ducts, leading to insulin deficiency in patients with cystic fibrosis. Hemochromatosis is linked to DM and insulin resistance [24].

viii) Endocrinopathies

Insulin resistance and increased hepatic glucose synthesis in peripheral tissues might induce or worsen underlying DM by increasing cortisol, growth hormones, adrenaline, and glucagon production [25, 26].

ix) Infections

Multiple infections have been associated with the onset of DM. One such infectious disease is congenital rubella. Approximately 20% of infants infected with the rubella virus develop type-1 DM later in life [27].

2.1.3. Pathophysiology

i) Insulin deficiency

Paul Langerhans discovered the cells known as "islets of Langerhans" in 1869, accounting for 1% of total pancreatic mass [28]. Insulin secretions from islets to reduce blood glucose levels were first described by Mayer and Schaefer in 1909 and 1910, respectively [29, 30]. Insulin is synthesized initially as a polypeptide precursor, pre-proinsulin. Later it quickly gets converted to proinsulin in the pancreas. Removing four amino acid residues produces equal quantities of insulin and C-peptide. Insulin holds 51 amino acids in two chains (‘A’ chain has 21 and ‘B’ chain has 30), which are joined by two disulphide bridges. Insulin and C-peptide (as well as some proinsulin) components are packed into granules in the islets. [31].

Glucose is the primary stimulator of insulin release. Both food intake and the release of gastrointestinal peptide hormones induce the reaction. The total amount of insulin secretion daily is around 40 units [31].

The liver and kidneys predominantly process the insulin and have a half-life of 3–5 minutes. Glomeruli filter insulin and tubules reabsorb insulin and degrade it in the kidneys [31].

An internal messenger cascade is started when insulin binds with a receptor on the cell surface and makes it possible for electrolytes, amino acids, and glucose to be transported.

In type 1 DM, acute insulin insufficiency leads to uncontrolled hepatic gluconeogenesis and glycogenolysis, which increases hepatic glucose production. In addition, glucose absorption in insulin-sensitive tissues, including adipose tissue and muscle, reduces hyperglycemia. When there is a metabolic imbalance, an infection, or another acute illness, the production of the counter-regulatory hormones cortisol,

catecholamine, growth hormone, and glucagon increases. All of them boost hepatic glucose synthesis even more [31].

Insulin production declines over time in people with type 2 DM. Hyperinsulinemia can keep glucose levels stable for a while, but ultimately, β-cell function deteriorates, resulting in hyperglycemia. Type 2 DM develops if this cycle does not break. Impaired glucose tolerance (IGT), impaired fasting glucose, or hyperinsulinemia may be identified prior to the onset of overt DM. If so, a rigorous diet and exercise program leading to weight reduction and increased insulin sensitivity may postpone or even prevent the formation of DM. Regardless of treatment, the β-cell function continues to deteriorate over time, necessitating frequent insulin therapy [31].

ii) Insulin resistance

Most patients with type 2 DM have a lot of abdominal fat, which is physiologically different from subcutaneous fat and can induce 'lipotoxicity.' The anti-lipolytic actions of insulin are resistant to abdominal fat, releasing large quantities of free fatty acids, preceding to insulin resistance in the liver and muscle. The results are an increase in gluconeogenesis in the liver and a reduction in insulin-mediated glucose uptake in the muscle. Both of them increase the glucose levels in the bloodstream. The buildup of fat in the liver, muscles, and pancreas, which causes insulin resistance in these organs, occurs when adipocytes become too large and cannot store more fat [31].

Excessive intra-cavity adipose tissue affects over exudation of specific cytokines (adipokines or adipocytokines) related to inflammation, endothelial dysfunction, and thrombosis. Plasminogen activator inhibitor-1 (a prothrombotic adipokine), tumour necrosis factor- and interleukin-6 (both proinflammatory adipokines), and resistin are examples of such adipokines (which cause insulin resistance). Hypercoagulability, poor fibrinolysis, a toxic mix of endothelial damage (produced by persistent subclinical inflammation), oxidative stress, and hyperglycemia induce atherosclerosis linked with insulin resistance. A beneficial adipokine, adiponectin, is secreted in low amounts by excess adipose tissue.

Adiponectin prevents monocytes from adhering to endothelial cells, which protects against vascular injury. Adiponectin levels are lower in people with type 2 DM than those without DM, and weight loss raises adiponectin levels [31].

iii) Pancreatic β-cells

Many of the same mechanisms (figure 2.1) that cause T2DM can also cause β-cell apoptosis, reducing β-cell mass and capacity to adjust insulin resistance (IR). Endoplasmic reticulum stress, oxidative stress, inflammatory cytokines, chronic hyperglycemia and chronic hyperlipidemia, are among the mechanisms [32]. The possible emerging mechanisms of β-cell dysfunction are discussed in table 2.2.

Table 2.2. Potential mechanisms of pancreatic β-cell dysfunction [33].

Glucotoxicity
Diminished insulin synthesis; augmented apoptosis; diminished intracellular insulin stores (degranulation); chronic oxidative stress, metabolic stress.
Lipotoxicity
Deficit of glucose-stimulated insulin secretion reaction; reduced insulin syntheses; metabolic stress; free fatty acid esterification; and cellular accumulation.
Increased requirement for secretion
Abnormal protein folding and endoplasmic reticulum strain
Amyloid accumulation
Increased absorption apoptosis and diminished insulin secretion.
Inflammatory progressions
Abnormal cytokine release from defective adipocytes; unusual immune activities at β-cells or in adipocytes; glucose-induced inflammatory process; autoimmune constituents.

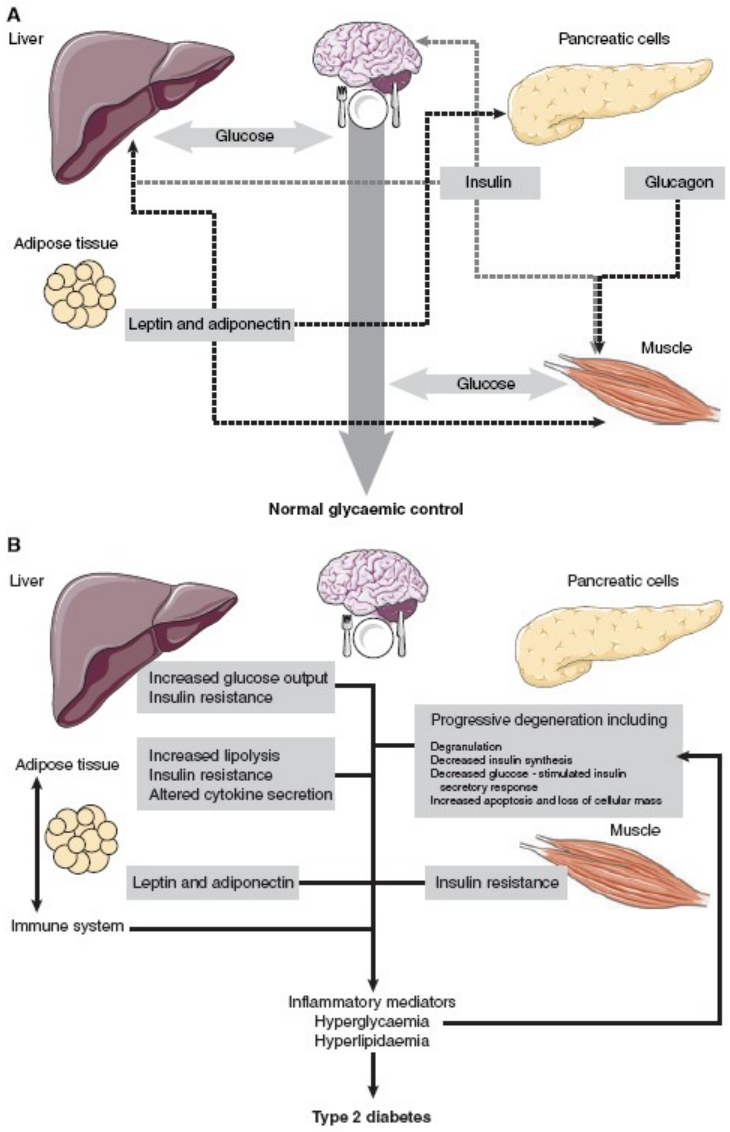


Figure 2.1. Normal metabolic regulation of glucose (A) and the underlying mechanisms in the development of Type-2 DM (B)
(Image adapted from Standl [34]).

2.1.4. Diagnosis of DM

HbA1c measures (table 2.3) are used in clinical procedures for the diagnosis of DM, according to the American Diabetes Association (ADA) [35, 36].

Table 2.3. ADA diagnostic criteria. [36]

Stage→	**Latent Period**	**Impaired glucose tolerance**	**Diabetic Period**
Criteria for diagnosing	Possessing at least two autoantibodies AND normal glucose levels	2-hour plasma glucose during OGTT: 140-199 mg/dl OR Fasting plasma glucose: 100-125 mg/dl OR HbA1C: 5.7-6.4%	2-hour plasma glucose during OGTT: ≥200 mg/dl OR Fasting plasma glucose: ≥126 mg/dl OR Random plasma glucose: 200 mg/dl when polyuria and weight loss are present. OR HbA1C ≥6.5%.

OGTT-Oral Glucose Tolerance Test, HbA1C-Measures amount of glucose attached to hemoglobin.

2.1.5. Signs and symptoms of DM

i) Warning signs

Irritability, unusual weight loss, dry mouth, numbness, burning pain, itching, reactive hypoglycemia, impotence, decreased vision, acanthoses nigricans, particularly in the urinary tract, genital area, oral cavity, and skin, and delayed wound healing are all warning signs [37].

ii) Classical signs and symptoms

Hyperglycemia is one of the most frequent signs of DM. Early DM is frequently asymptomatic. More severe hyperglycemia causes glycosuria, which leads to increased urine frequency, osmotic diuresis, polydipsia, and polyuria, all of which may contribute to dehydration and orthostatic hypotension. Dehydration causes fatigue, weakness, and mental disturbances. Because plasma glucose levels fluctuate, symptoms may appear and go. Polyphagia is a sign of hyperglycemia that is not usually a significant source of worry for patients. Nausea, weight loss,

impaired vision, and vomiting are all symptoms of hyperglycemia, which can also make one more susceptible to bacterial and fungal infections [15, 38].

Patients with type-1 DM often have symptomatic hyperglycemia and sometimes diabetic ketoacidosis (DKA) [15, 38].

Patients with type-2 DM may have clinical hyperglycemia, although they are frequently asymptomatic, and their disease is first detected via regular testing. In some people, hyperosmolar hyperglycemia develops first, especially during stress or when drugs like corticosteroids further decrease glucose metabolism [15, 38].

2.1.6. Risk factors for DM

Epidemiological data from India have shown the following major risk factors for DM [38, 39].

- Activist family history of DM
- Age > 35 yr
- Overweight and obesity
- Enlarged waist (For males, 90 cm, and for women, 80 cm)
- Hypertension
- Inactive lifestyle
- Gestational DM

2.1.7. Treatment for DM

Insulin is used to treat type-1 diabetes, while a variety of oral agents belonging to distinct pharmacologic families, each with its mechanism of action, side effect profile, and toxicity, are available to treat T2DM [40]. The mechanisms, glycemic effects, general features, PK properties of antidiabetic drugs (table 2.4 & 2.5) and insulin preparations (table 2.6) are shown below.

Table 2.4. Oral antidiabetic drugs along with their mechanism of action [40]

Class	Mechanisms	Drugs
Sulfonylureas	Insulin secretion increases. Pancreatic β-cell arousal	***First generation:*** Tolbutamide, acetohexamide, tolazamide, chlorpropamide ***Second generation:*** Glimepride, gliquidine, glibenclamide, gliclazine
Meglitinides Phenylalanines	The production of insulin rises. Pancreatic β-cell arousal	Nateglinide and Repaglinide
Biguanides	The synthesis of hepatic glucose is decreases. Reduce glucose absorption in the intestine Elevates the glucose uptake in the peripheral tissues. Insulin sensitivity will improve	Metformin
Thiazolidinediones	Enhance insulin sensitivity. Conserve β-cell role May reinforce β-cells	Rosiglitazone and pioglitazone
α-glucosidase inhibitors	Interruption in carbohydrate breakdown	Miglitol and acarbose
Dipeptidyl peptidase-4 inhibitors	Upsurge insulin secretion from the pancreas Restrains glucagon Reduces inactivation of GIP & GLP-1	Vildagliptin, sitagliptin, saxagliptin

Table 2.5. Newer antidiabetic drugs along their mechanisms of action [40]

Agents	The Principle Mechanism of Action
GLP-1 analogs[a]	Increased satiety, and delays stomach emptying, arouses glucose-dependent insulin secretion and inhibits glucagon secretion.
DPP-4 inhibitors[b]	Glucagon production suppresses; the stimulation of glucose-dependent insulin secretion.
Pramlintide[a]	Glucagon secretion is suppressed, increases satiety, and slows stomach emptying.
Rimonabant[b]	It prevents CB1 receptors, diminishes appetite, and decreases lipogenesis
a-Parenteral; b-Oral	

Table 2.6. Insulin preparations for Type 1 and Type 2 DM [41].

Type & Preparation	Components	Action Summary (h)		
		Onset	Peak	Duration
Ultra rapid acting				
Lispro (human analog)	Identical to standard human insulin except for the presence of transposed proline and lysine in the β chain.	0.2-0.5	0.5-2	3-4
Short-acting				
Regular (human)	Unaltered zinc insulin crystal solution	0.5-1	2-3	6-8
U-500 (pork)	Concentrated unaltered	1-3	6-12	12-18
Intermediate-acting				
Lente (human)	Amorphous acetate buffer	1.5-3	7-15	16-24
NPH (human)	Zinc, phosphate buffer, protamine	1.5	4-10	16-24
Long-acting				
Ultralente (human)	Crystalline mix and amorphous	3-4	9-15	22-28
Mixtures (human)				
50/50	NPH 50%, regular 50%	0.5-1	2-12	16-24
70/30	Regular 30%, NPH 70%,	0.5-1	3-12	16-24
NPH-neutral protamine hagedorn				

Type-2 DM is a complex illness with a variety of underlying pathophysiological mechanisms. As a result, it should tailor treatment to the severity of hyperglycemia, hyperinsulinemia, or insulin insufficiency. In addition, many considerations must be considered while prescribing a certain medicinal drug. Efficacy, safety, cost and simplicity of administration are among these variables. The various treatment approaches for type-2 DM are depicted below (figure 2.2):

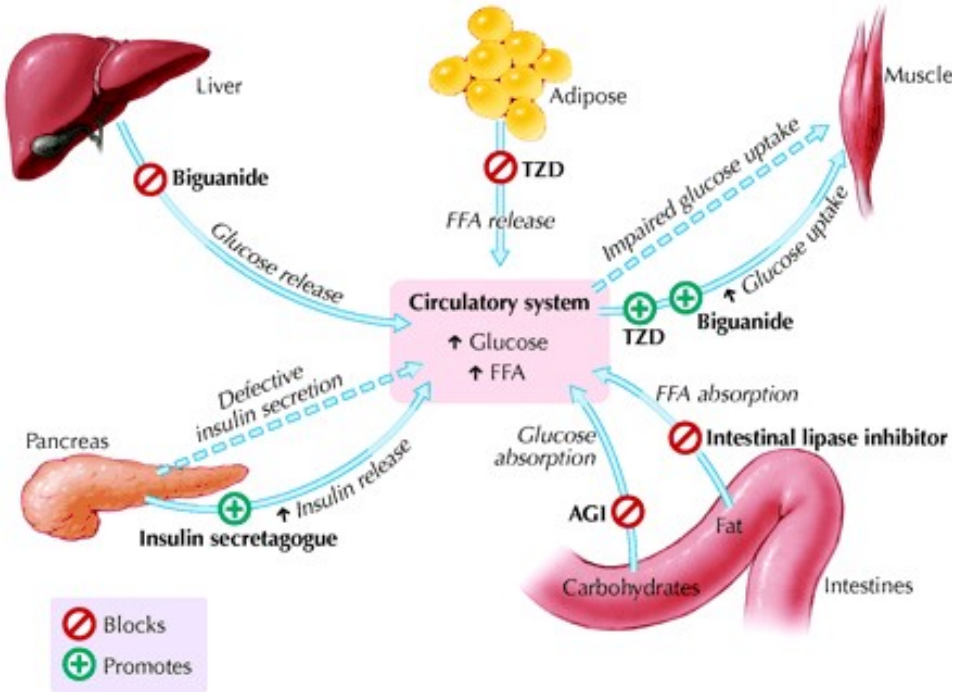


Figure 2.2. Major targets and actions of orally administered anti-diabetic drugs

(*Adapted from Cheng et al. [42]).*

AGI, α-glucosidase inhibitor; FFA, free fatty acid; TZD, thiazolidinedione

2.2. Herb-Drug Interaction (HDI)

HDI is defined as any alteration in a drug's PK and/or PD due to the concomitant administration of a herb/herbal product. HDIs may enhance or reduce drug activity or result in drug-related toxicity [43]. Thus, HDIs can be a double-edged sword, posing both dangers (adverse drug effects) and advantages (enhancing performance).

2.2.1. Types of HDIs

The primary cause of HDIs is the intersecting substrate specificity in the bio-transformational paths of the physiological systems [44]. HDIs can be categorized as PD-HDIs or PK-HDIs.

2.2.1.1 PD-HDIs

PD interactions may take place at three levels: (a) the receptor, (b) the signalling level (2nd messenger), and (c) the effector level (figure 2.3) [45]. PD-HDIs can arise because of attractions for common receptor sites, which can cause synergistic or additive effects of herbal medicines with conventional pharmaceuticals [46]. These effects may result in PD toxicity or antagonistic reactions (table 2.7). The PD phase of drug action, as per AriEns and Beld [47] is the interaction of the drug fragment with its molecular targets, which initiates a response that results in a change in the measured parameters and is deemed as a consequence through a set of phases involving transduction, overexpression, and modulation. Elevated transaminases [48, 49], acute and chronic hepatitis [50, 51], liver failure [52], veno-occlusive disorders [53], liver cirrhosis [54], fibrosis [55], cholestasis [56], zonal or diffusive hepatic necrosis [57], and steatosis [58] are all examples of liver injury inducible by phytochemical agents. Table 2.8 enlists PD-HDIs reported in scientific literature.

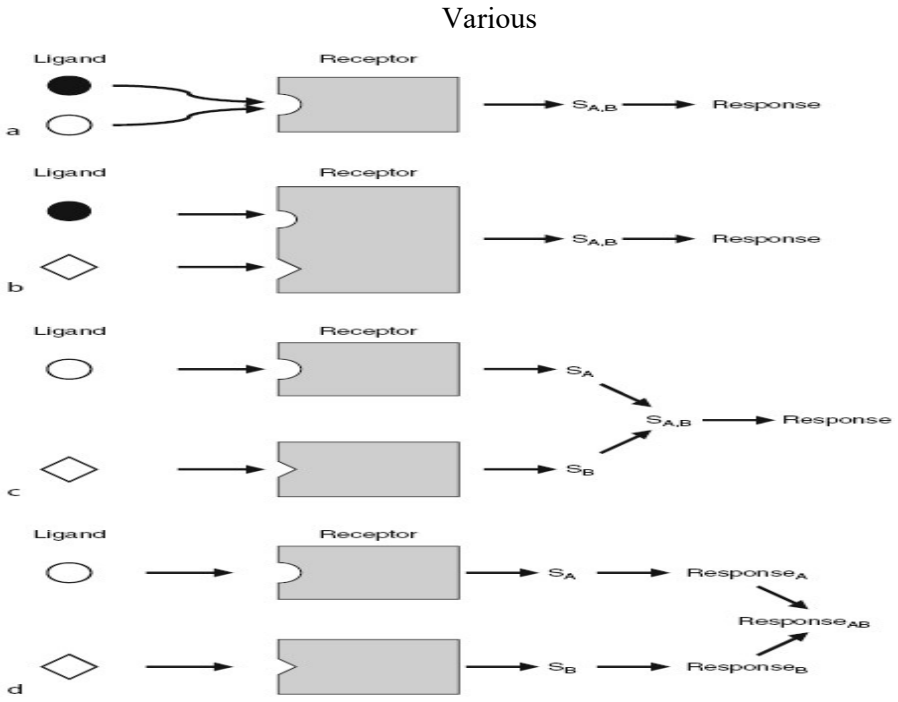


Figure 2.3. Mechanisms underlying PD drug-drug interactions *[45]*
a-at receptor; b-signaling (e.g., 2nd messenger); or c&d-effector levels; SA-Substance A; SB- Substance B

Table 2.7. Consequences of PD-HDIs [59].

Effect	**Both agents are effective individually**	**Only one agent is effective individually**	**Neither agent is effective individually**
The impact of the combination is more than expected.	Synergistic effect	Synergistic effect	Coalism
The impact of the combination is the same as expected.	Effects' independence and additivity	Inertism	Inertism
The impact of the combination is less than expected.	Antagonistic effect	Antagonistic effect	—

Table 2.8. PD interactions between herbs/herbal products and allopathic drugs

Herb/Herbal Product	Allopathic drug	Mechanism of Action	Consequences	References
Aloe *(Aloe vera)*	Sevoflurane	An additive impact influences platelet function.	Blood loss	60
Chamomile *(Matricaria recutita)*	Warfarin	Coumarins, which are found in chamomile, may have anticoagulant properties.	Bleeding	61
Betel nut *(Arecha catechu)*	Procyclidine	Arecoline from betel nut has an antagonistic effect on the anticholinergic drug procyclidine.	Bradykinesia, rigidity, jaw tremors	62
Boldo *(Peumus boldus)*	Warfarin	According to new research, Boldo may increase bleeding time in people using warfarin.	Anticoagulant action is enhanced.	63
Chlorella *(Chlorella pyrenoidosa)*	Warfarin	Chlorella is high in vitamin K. It may reduce warfarin's anticoagulant impact.	Anticoagulant action is reduced.	64
Cat's claw *(Uncaria tomentosa)*	Ritonavir, atazanavir & saquinavir	The protease inhibitors ritonavir, atazanavir, saquinavir all showed increased serum trough concentrations. The likelihood of medication interaction is high, according to the Horn drug interaction scale. Future studies must identify the mechanism.	Increased protease inhibitor concentration	65
Danshen *(Salvia milthiorriza)*	Warfarin	An additive coagulation effect	Anticoagulant action is enhanced.	66
Evening primrose *(Oenothera biennis)*	Fluphenazine	Evening primrose oil includes GABA, which may lower the seizure threshold.	Seizures	67
Fenugreek	Warfarin	Fenugreek lengthened bleeding time.	Enhanced	68

Herb/Herbal Product	Allopathic drug	Mechanism of Action	Consequences	References
(Trigonella foenum-graecum)			anticoagulant effect	
Garlic *(Allium sativum)*	Fluindione	The mechanism remains unknown.	Fluindione's anticoagulant impact has been reduced.	69
Ginger *(Zingiber officinale)*	Phenprocoumon	Under long-term phenprocoumon treatment, the interaction increased INR and epistaxis (phenprocoumon is a warfarin analog). Ginger preparations have been proven to have significant antiplatelet effects.	Enhanced anticoagulant activity	70
Ginkgo *(Ginkgo biloba)*	valproic acid and phenytoin	After a fatal incident, both anticonvulsants combined with ginkgo were found at subtherapeutic plasma levels, according to an autopsy.	Fatal seizure	71
Ginseng (Korean ginseng)	Imatinib	According to the Horn drug interaction scale, the chance of a medication interaction was high; the mechanism is unclear.	Hepatotoxicity	72
Goji Chinese wolfberry *(Lycium barbarum)*	Warfarin	An instance, a re-challenge verified an interaction. The mechanism remains a mystery.	Enhanced anticoagulant effect	73
Green tea *(Camellia sinensis)*	Warfarin	Green tea's vitamin K may counteract warfarin's effects.	Anticoagulant action is reduced.	74
Kava *(Piper methysticum)*	Alprazolam	This interaction may be due to an additive effect on GABA receptors; however, there was insufficient evidence in the report to estimate the	State of drowsiness and disorientation	75

Herb/Herbal Product	Allopathic drug	Mechanism of Action	Consequences	References
		plausibility of the interaction.		
Kava *(Piper methysticum)*	Levodopa	A dopamine antagonistic relationship explains this interaction.	Decreased efficacy of levodopa	76
Mistletoe *(Viscum album)*	Busulphan	The mechanism is unclear	Fibrosis of the organs and death	77
Passion flower *(Passiflora incarnate)*	Lorazepam	Passion flowers may increase lorazepam's CNS-depressant impact.	Dizziness, hand tremor, muscular fatigue, and throbbing	78
Prickly pear cactus *(Opuntia polyacantha)*	Metformin and glipizide	An additive hypoglycemic impact may explain an interaction.	Hypoglycaemic effect	79
St. John's wort *(H. perforatum)*	Buspirone	The interaction explains a cumulative impact on 5-HT reuptake.	Serotonin syndrome and hypomanic episode	80
Valerian *(Valeriana officinalis)*	Loperamide	The mechanism is unknown	Acute delirium	81
Valerian *(Valeriana officinalis)*	Lorazepam	A depressive impact on the central nervous system is additive	Dizziness, hand tremor, muscular fatigue, and throbbing	82

2.2.1.2. PK HDIs

Generally, PK drug interactions are associated with modifications in drug absorption (rate and extent), distribution (displacement of plasma protein binding), metabolism (either induction or inhibition), and excretion (renal, pulmonary, and biliary). PK drug interactions are explained by modified absorption, intervention in distribution, and modifications and competition in metabolism and excretion pathways [83]. Potential mechanisms underlying PK drug interactions are listed as follows:

i) Metabolic enzyme induction and Inhibition
ii)Inhibition or induction of transport and efflux proteins
iii) Alteration of gastrointestinal functions
iv) Alteration in the renal elimination

i) Metabolic enzyme induction and inhibition

The CYP superfamily is engaged in the bioconversion of xenobiotics and endogenic chemicals via peroxidative, oxidative, and reductive pathways [84, 85]. CYP from families one, two, and three are largely involved in metabolism, whereas others are involved in the hormone, bile acid, and fatty acid synthesis and removal process [86]. The majority of CYP from families 1, 2, and 3 are involved in the metabolism of xenobiotics, while others are involved in the synthesis and removal of endogenous compounds such bile acids, hormones, and fatty acids [87, 88]. 3A5, 1A2, 3A4, 2A6, 2E1, 2C9, 2D6, 2C19 and 2D6 are the most significant CYP subfamilies in human drug metabolism [89, 90].

Two primary associates of the human CYP1A subfamily are 1A1 and 1A2. Extrahepatic tissues such as the kidneys, gut, and lungs express CYP 1A1, while CYP1A2 accounts for around 15% of overall liver CYP [91]. Although most CYP2B subfamily members conduct minor roles in metabolism, CYP2B6 is involved in medication metabolism [92]. Subfamily 2C is the 2nd most frequent CYP in the human liver after subfamily 3A, accounting for around 20% of all CYP. It contains three active players: 2C9, 2C8, and 2C19, all engaged in retinol and retinoic acid metabolism [93]. Chlorzoxazone, paracetamol, and enflurane are the three medicines that are processed by CYP2E1, the most active member of the 2E subfamily [94].

CYP3A4 is the most prevalent isoform, abundantly conveyed in the liver and gut, and implicated in 50% of all drug metabolisms. Over 40% of human CYP is produced by the CYP3A4 subfamily, with CYP3A4 being the most abundant of all isoforms, being strongly expressed in the liver and intestines, and being involved in the metabolism of almost half of all currently used authorised medicines [95, 96]. The selectivity and specificity of inhibitors and substrates for these enzymes are helpful in toxicological and PK studies. In toxicological and PK research, the selectivity and specificity of inhibitors and substrates for these enzymes are useful.

a) Induction

Enhanced mRNA transcription results in greater protein intake levels than average physiological values, resulting in increased intestinal and hepatic enzyme activity. When this happens, it speeds up the drug metabolism, impacting the drug's oral bioavailability and systemic distribution. Pre-systemic metabolism is frequently addressed in oral medicines' composition and dosage planning to obtain predictable systemic bioavailability. If this equilibrium is disturbed, blood drug concentrations may change significantly. Many herbal compounds induced CYP. Lower therapeutic plasma levels might arise from concurrent use of enzyme-inducing herbal medicines and prescription drugs, which could result in therapeutic failure [86].

b) Inhibition

CYP and many other metabolic enzyme inhibitions are generally competitive, leading to immediate, concentration-dependent effects [97]. Most inhibitors are also CYP substrates [98]. The PK profiles of xenobiotics are considerably altered due to this occurrence. Because of the projected pre-systemic gastrointestinal and liver metabolism restriction and xenobiotic plasma levels are unusually high. This observation's ultimate impact might be a toxic manifestation. Due to reduced hepatic clearance, drug buildup is another clinically significant side effect of enzyme inhibition. These adverse effects will be particularly troublesome for medications with a narrow therapeutic range or abrupt dose-response curves [86]. Table 2.9 lists several herbal medicines and their phytochemical components that can interact with CYP.

Table 2.9. PK interactions between herbs/herbal products and allopathic drugs (CYP mediated)

Herb/Herbal Product	Allopathic drug	Mechanism	Consequences	References
Danshen *(Salvia milthiorriza)*	Midazolam	Induction of CYP3A4	Increased blood concentrations of midazolam	99
Garlic *(Allium sativum)*	Chlorzoxazone	Garlic inhibits the CYP2E1 enzyme that metabolizes chlorzoxazone.	Chlorzoxazone ratios have decreased.	100, 101
Garlic *(Allium sativum)*	Paracetamol	The PKs of paracetamol are altered by garlic. This interaction is unlikely to have any clinical implications.	Variations in paracetamol PKs	102
Garlic *(Allium sativum)*	Saquinavir	Garlic may increase P-glycoprotein levels in the intestines, lowering saquinavir absorption.	Reduced saquinavir levels in the blood	103
Ginkgo *(Ginkgo biloba)*	Efavirenz	Biological tests demonstrated a decrease in efavirenz concentration, corresponding to a rise in viral load. It's essential to sort out how it works.	Virologic failure is caused by decreased levels of efavirenz in the blood	104
Ginkgo *(Ginkgo biloba)*	Risperidone	Priapism may be a significant side effect of antipsychotic medicines in rare cases. Interaction confirmation is necessary. Both ginkgo and risperidone can dilate blood vessels.	Priapism	105
Goldenseal *(Hydrastis canadensis)*	Debrisoquine	This interaction suggests that CYP2D6 is being inhibited.	The urine recovery ratio of debrisoquine has	106

Herb/Herbal Product	Allopathic drug	Mechanism	Consequences	References
			reduced	
Goldenseal *(Hydrastis canadensis)*	Midazolam	This interaction suggests that CYP3A4 is being inhibited.	Increased blood levels of midazolam	107
Green tea *(C. Sinensis)*	Simvastatin	Green tea and simvastatin interact strongly since drinking green tea raise plasma concentration of simvastatin acid and simvastatin lactone. Unknown is the mechanism behind such interaction. On the enzyme CYP3A4 that deteriorates simvastatin, green tea has no effect.	Increased simvastatin blood levels are linked to statin sensitivity	108
Hibiscus *(Hibiscus sabdariffa)*	Paracetamol	The paracetamol PK characteristics were affected by Zobo beverage; sweet water extricated the dried calyx of *Hibiscus sabdariffa*. The clinical implications of such an interaction are unknown.	Changes in some paracetamol PK parameters	109
Kava *(Piper methysticum)*	Chlorzoxazone	Kava may inhibit the CYP2E1 probe chlorzoxazone.	Chlorzoxazone serum ratios have decreased	107
Milk thistle *(Silybum marianum)*	Metronidazole	CYP3A4 and CYP2C9 also metabolize P-glycoprotein substrate metronidazole. In studies, silymarin lowered the serum levels and AUC of metronidazole.	Metronidazole blood concentration has reduced	110
Peppermint *(Mentha piperita)*	Felopidine	The Cmax and AUC of felodipine and its metabolite dehydrofelodipine were both raised by peppermint oil. Menthyl acetate and menthol, the major components of peppermint oil, were modestly reversible inhibitors of CYP3A4. As a result, the interaction is most likely	Increased felopidine concentration in the blood	111

Herb/Herbal Product	Allopathic drug	Mechanism	Consequences	References
		mediated by inhibition of CYP3A4.		
Red yeast rice	Cyclosporine	Natural statins (CYP3A4 substrates) are found in red yeast rice, and cyclosporine might conceivably enhance their blood levels (CYP3A4 inhibitor). If validated, this is a mechanism through which cyclosporine increases the toxicity of a herbal substance (i.e., red-yeast rice).	Rhabdomyolysis	112
Schisandra Chinensis	Talinolol	The herbal extract *S. Chinensis* substantially raised the talinolol AUC and T_{max} of talinolol, indicating that it inhibited P-glycoprotein in humans.	Increased talinolol concentration in the blood	113
Schisandra sphenanthera	Tacrolimus	In China, patients undergoing kidney and liver transplants are often treated with tacrolimus and Schisandra sphenanthera. Schisandra sphenanthera inhibits CYP3A4 activity.	Increased tacrolimus concentration in the blood	114, 115
Schisandra sphenanthera	Midazolam	Midazolam oral bioavailability was improved by *S. sphenanthera.*	Increased blood concentrations of midazolam	116
St. John's wort *(Hypericum perforatum)*	Amitriptyline	The interaction is most likely mediated via CYP3A4 and P-glycoprotein induction.	Amitriptyline levels in the blood have reduced.	117
	Atorvastatin	The interaction is most likely mediated via CYP3A4 and P-glycoprotein induction.	Atorvastatin's effectiveness has been reduced.	118
	Chlorzoxazone	St. John's wort improved the chlorzoxazone serum ratios in both clinical studies, suggesting CYP2E1 induction.	Chlorzoxazone serum ratio rises.	119, 120

Herb/Herbal Product	Allopathic drug	Mechanism	Consequences	References
	Cyclosporine	The induction of CYP3A4 and P-gp by St. John's wort is the most likely cause.	Cyclosporine levels in the blood have declined.	121

ii) Inhibition or induction of transport and efflux proteins

Drug transporters belonging to the ATP binding cassette (ABC) family play an essential role in penetration, diffusion, and excretion. The human MDRI gene encodes P-gp, a 170-kDa. It may be found on the columnar mucosal cells (of the colon, gut, and adrenal glands) as well as the apical epithelia of the bile canaliculi of the liver, the proximal tubules of the kidneys, the pancreatic cells, and many other body tissues [122, 123]. It helps the intestine, liver, renal, and brain absorb and eliminate drugs. These proteins excrete drugs and their metabolites via the hepatobiliary, direct intestinal, and urinary systems [124]. Thus, the effect of a co-administered herb on P-gp can change the drug's PK profile.

Herbal medications, via a competitive or non-competitive mechanism, block or reduce drug transporters' typical activity level, resulting in PK interaction. Interactions can also occur when transport proteins are induced by increasing the relevant protein's mRNA. Phytochemicals, such as quinidine, flavonoids, reserpine, furanocoumarins, yohimbine, vinblastine, and vincristine, found therapeutically significant P-gp inhibitors in studies. Mobile ionophores including nigericin, valinomycin, monensin, nonactin, lasalocid, and calcimycin have been shown to block anthracycline efflux through P-gp [125, 126, 127, 128, 129, 130, 131], but channel-forming ionophores like gramicidin do not stop this [132]. Various herbal compounds that interact with CYP also affect transport proteins similarly. Table 2.10 shows the effects of several herbs on transport proteins.

Table 2.10. PK interactions (transporter protein-mediated) between herbs/herbal products and allopathic drugs

Herb/Herbal products	Drug transporter	Drug candidates	References
Rosmarinus officinalis	P-glycoprotein (ABCB-1, MDR-1)	Actinomycin D, mitoxantrone, Daunorubicin, teniposide, docetaxel, etoposide, irinotecan, paclitaxel, topotecan, vincristine, vinblastine, mitomycin C, tamoxifen, epirubicin, bisantrene, tipifarnib, doxorubicin	[133, 134]
Curcuma longa	MRP-1 (ABCC-1)	Etoposide, vincristine, teniposide, vinblastine, daunorubicin, epirubicin, doxorubicin, topotecan, idarubicin, mitoxantrone, irinotecan, methotrexate, melphalan, chlorambucil.	[135]
Inchin-ko-to	MRP-2 (ABCC-2)	Methotrexate, SN-38G (metabolite of irinotecan), vinblastine, sulfinpyrazone	[136]

Herb/Herbal products	Drug transporter	Drug candidates	References
Herbs include Gymnema Sylvestre, Glycine Max (soybean), and Cimicifuga racemosa that contain flavonoids (black-cohosh)	BCRP (ABCG-2, MXR)	9-Aminocamptothecin, epirubicin, daunorubicin, lurtotecan, mitoxantrone, etoposide, topotecan, SN-38	[137, 138]

ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; MRP, multidrug resistance-associated protein; MDR, multidrug resistance gene; MXR, mitoxantrone resistance-associated protein.

iii) Alteration of gastrointestinal functions

Dissolution characteristics and absorption of pH-dependent medicines like itraconazole and ketoconazole may be affected by changes in abdominal pH and biochemical factors. Chelation and complex formation, which lead to insoluble tough compounds and rivalry at the absorption site, may substantially influence medication absorption, particularly with the site-specific formulation. Anthranoids in plants like Cascara, Cassia, and Rhubarb and soluble fibres like psyllium and guar gum may help minimize medication absorption by shortening GI transit time. It has been shown to enhance the motility of the GIT. Because of the reduced GI transit time when used with prescribed medicine, substantial changes in the latter's absorption have been documented [139].

According to Izzo et al., anthracoid harm the intestinal epithelium because they impede Na+/K+ ATPase and upsurge the nitric oxide synthase activity. The difference in water and salt absorption in the intestines and the consequent fluid accumulation resulted in a significant rise in intestinal transit [140]. In the rat's GIT, Munday and Munday observed that a garlic-derived compound boosted tissue functions of glutathione transferase and quinone reductase. Because of their metabolic processes, both enzymes are thought to be chemoprotective, especially against chemical carcinogens [141]. Ginseng's well-known PK HDI may be caused by its GI effects, specifically its suppressive effects on GI secretion, in addition to P-gp and CYP-mediated pathways [142]. Danthron and rhein have been found in vitro to improve furosemide uptake, and a water-soluble medicine is used once a week [143]. Polygonum paleaceum, a Chinese medicinal plant, has been shown in animal studies to lower gastrointestinal motility, suppress bowel reflexes, and delay stomach emptying [144]. The investigation discovered the effects of the two Chinese herbal medicines, Radix paeniae alba and Fructus aurantii immature, on GI motility [145].

By sequestering bile acids, high-fibre herbal products may reduce the absorption of medications, including glibenclamide, metformin, phenoxymethylpenicillin, and lovastatin [146]. Mochiki et al. found that Kampo, a traditional Japanese drug, can enhance the production of gastrointestinal hormones such as motilin, calcitonin gene-related peptide, and vasoactive intestinal peptide

[147]. Ghrelin, a hunger-related hormone, increased intestinal secretion, causing stomach emptying to be delayed [148, 149, 150, 151]. Furthermore, Qi et al. discovered that Da-Cheng-Qi-Tang, an herbal drug, may raise plasma motilin, augment GI motility, reduce gastroparesis, and treat gastric dysrhythmia after surgical treatment [152]. These effects can reduce the amount of time a medication spends in the intestine, resulting in less absorption.

iv) Alteration in the renal elimination

Herbal substances that affect renal function may alter the kidneys' ability to eliminate medications. This interaction may be caused by tubular secretion inhibition, reabsorption interference, or glomerular filtration interruption [153]. Herbal products in this category are used as diuretics. Herbal diuresis is a complicated and variable procedure. Some herbs boost glomerular filtration but not electrolytes, whereas others irritate the tubules directly [154, 155]. Table 2.11 lists several herbs that can interfere with renal functions and drug excretion.

Table 2.11. Herbs/herbal products capable of PK interactions (mediated through variation in renal functions)

Herb/Herbal product	Brief description	Mechanism	References
Aristolochia fangchi	Chinese slimming herbal remedy	In renal tissues, the presence of aristolochic acid causes DNA adducts that significantly reduce the number of cortical tubules	[156]
Impila (*Callilepis laureola*)	Popular medicinal plant from South Africa	It is hepatotoxic and damages the loop of Henle and the proximal convoluted tubules	[157]
Djenkol bean (*Pithecellobium lobatum*)	African medicine uses for a pungent fragrance.	Comprises nephrotoxic djenkolic acid	[158, 159]

Herb/Herbal product	Brief description	Mechanism	References
Licorice root (*Glycyrrhiza glabra*)	Roots and extracts of a leguminous plant (of Asia and Europe) are used to treat chronic hepatitis and other conditions.	It contains glycyrrhizic acid, whose metabolite, glycyrrhetinic acid, inhibits renal 11-hydroxysteroid dehydrogenase, causing cortisol to build up in the kidneys and stimulating aldosterone receptors in cortical cells, which increases blood pressure, causes salt retention and causes hypokalemia. Drugs like digoxin may have their effects enhanced as a result of this.	[160, 161]
Wild mushrooms	Widely eaten in Africa	Nephrotoxic orellanine is present in certain species, most notably Cortinarius.	[162]
Rhubarb (*Rheum officinale*)	Used as laxative	Renal diseases such as renal stone production may be accelerated by high oxalic acid concentration.	[163]
Alfalfa (*Medicago sativa*), Noni fruit (*Morinda citrifolia*), Horsetail (Equisetum arvense), Dandelion (*Taraxacum officinale*), stinging nettle (*Urtica dioica*).	Used as traditional remedy in various ways, and studies have revealed that their extracts have unusually high potassium levels.	Hepatotoxic, hyperkalemic,	[164, 165, 166]
Goldenrod (Solidago virgaurea), Uva ursi	The diuretic properties of certain	Plants contain diuretic properties, which may help	[167, 168]

Herb/Herbal product	Brief description	Mechanism	References
(*Arctostaphylos uva ursi*), juniper berry (*Juniperus communis*), dandelion (*Taraxacum officinale*), lovage root (*Levisticum officinale*), horsetail (*Equisetum arvense*), asparagus root (*Asparagus Officinalis*), parsley (*Petroselinum crispum*).	plants	other medications be eliminated more quickly via the kidneys.	
Star fruit (*Averrhoa carambola*)	In Southeast Asia and South America, trees are often employed as antibacterial and antioxidant agents.	Oxalate nephropathy	[169, 170]

2.3. DRUG PROFILES

2.3.1. Repaglinide

Repaglinide is a medication used to treat type 2 DM. It is a member of the meglitinide class of antihyperglycemic medications.

2.3.1.1. Physicochemical properties of Repaglinide

Table 2.12. Physicochemical properties of Repaglinide [171]

Parameter	**Properties**
IUPAC name	2-ethoxy-4-({[(1S)-3-methyl-1-[2-(piperidin-1-yl)phenyl]butyl]carbamoyl}methyl)benzoic acid
Common name	Repaglinide
Molecular formula	$C_{27}H_{36}N_2O_4$
Appearance	White crystalline powder, odourless.
Molecular weight	452.6 g/mol
Density	1.137±0.06 g/cm^3
Melting point	129-130.2°C
Boiling point	672.9±55.0°C
Solubility	Soluble in water 89.99mg/L at 25ºC and soluble in Dimethyl sulfoxide: 34mg/ml
pKa	4.19±0.10
Storage temperature	2-8°C

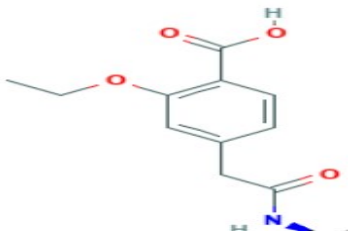


Figure 2.4. Chemical Structure of Repaglinide [171]

2.3.1.2. PD Properties of Repaglinide [172]

- **Drug class**: Meglitinide antidiabetics
- **Indication:** For the therapy of non-insulin-dependent DM (NIDDM) or T2DM.
- **Mechanism of action:** Repaglinide decreases blood glucose levels by increasing pancreatic insulin secretion. This action depends on pancreatic islets' functioning beta (β) cells. Insulin release is glucose-dependent and diminishes at low glucose concentrations. Repaglinide closes ATP-dependent potassium channels in the β-cell membrane by binding at characterizable sites. This potassium channel blockade depolarizes the β-cell, which leads to an opening of calcium channels. The resulting increased calcium influx induces insulin secretion. The ion channel mechanism is highly selective, with a low affinity for heart and skeletal muscle.
- **Dosage:** 0.5mg, 1mg, and 2mg are available in the market. Maximum 16 mg/day PO for adults and elders.
- **Drug-Drug interactions**: CYP2C8 is a substrate of repaglinide and can produce hypoglycemia when abiraterone inhibits CYP2C8. Repaglinide is a CYP3A4 substrate that can produce hyperglycemia owing to its stimulating action on CP3A4 (acetaminophen) and hypoglycemia because amoxicillin inhibits CYP3A4. β-blockers prevent catecholamines from being released by blocking β-receptors in the pancreas, resulting in hyperglycemia. Epinephrine and other sympathomimetics enhance hepatic glucose synthesis and glycogenolysis while inhibiting insulin secretion, resulting in hyperglycemia, via stimulating α and β receptors.
- **Significant adverse reactions:** Arrhythmia, myocardial infarction, anaphylactoid, heart, pancreatitis, hemolytic anemia, Stevens-Johnson syndrome, hypoglycemia, constipation, chest pain, angina, hypertension, thrombocytopenia, leukopenia, elevated hepatic enzymes, confusion, palpitations, sinus tachycardia, hepatitis, jaundice, peripheral edema, infection,

headache, back pain, dyspepsia, diarrhea, nausea, sinusitis, arthralgia, vomiting, paresthesias tremor, pallor, alopecia, weight gain.

2.3.1.3. PK properties of Repaglinide [173]

- **Absorption:** Repaglinide is rapidly and fully absorbed after oral treatment. The absolute bioavailability is around 56%. The highest biological effect is shown in 3-3.5 hours, and plasma-insulin levels stay high for 4-6 hours. The area under the curve (AUC) for a 2 mg repaglinide dosage in healthy people is 18.0 - 18.7 (ng/mL/h)^3.
- **Volume of distribution:** Repaglinide has a volume of distribution of 31 L after IV treatment for healthy people.
- **Protein binding:** Protein binding of repaglinide is >98% (e.g. α1-acid glycoprotein and albumin).
- **Metabolism:** CYP450 and 2C8 quickly metabolize repaglinide through oxidation and de-alkylation to form the most important dicarboxylic corrosive derivative. The aromatic amine derivative is also produced by oxidation. The carboxylic acid group of repaglinide is glucuronidated to produce an acyl glucuronide. The metabolites of repaglinide do not have much hypoglycemic action.
- **Elimination**: In 90% of cases, excretion of repaglinide is via feces (2% as an unaltered drug) and 8% via urine (0.1% as an unchanged drug).
- **Half-life:** The half-life of repaglinide is 1 hour.
- **Clearance:** Renal clearance of repaglinide is 33-38 L/hour following IV administration.
- **Toxicity:** LD_{50} >1 g/kg (rat)

2.3.2. Sitagliptin

Sitagliptin is used to manage hyperglycemia caused by type-2 DM and belongs to dipeptidyl peptidase-4 (DPP-4) inhibitors [174].

2.3.2.1. Physicochemical properties of Sitagliptin [174]

Table 2.13. Physicochemical properties of Sitagliptin

Parameter	**Properties**
IUPAC name	(3R)-3-amino-1-[3-(trifluoromethyl)-5H,6H,7H,8H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one
Common name	Sitagliptin Phosphate
Molecular formula	$C_{16}H_{15}F_6N_5O$
Appearance	White solid
Molecular weight	407.32 g/mol
Density	1.61±0.1g/cm^3
Melting point	114.1-115.7^{o}C
Boiling point	529.9±60.0 ^{o}C
Solubility	Soluble in water, 179.2 mg/L at 25°C
pKa	7.20±0.10
Storage temperature	20-25°C

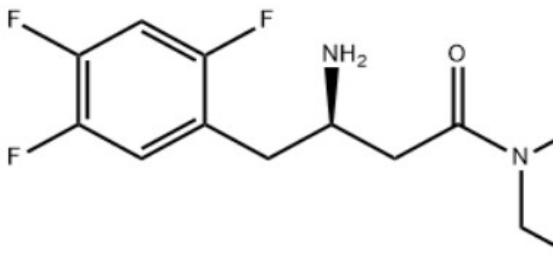


Figure 2.5. Chemical structure of Sitagliptin [174]

2.3.2.2. PD properties of Sitagliptin [175]

- **Drug Class:** Dipeptidyl Peptidase-4 (DPP-4) Inhibitor Antidiabetics
- **Indications:** Non-insulin-dependent DM (NIDDM) or T2DM
- **Mechanism of action:** Sitagliptin increases the proteins activity that enhance insulin release when blood sugar levels rise (e.g. after a meal). Sitagliptin is a DPP-4 inhibitor enzyme which metabolizes the incretin hormones GLP-1 and GIP, resulting in increased pancreatic glucose-dependent insulin release and reduced hepatic glucose production. Limiting DPP-IV activity would prolong and amplify the effects of GLP-1 by slowing down the rate of GLP-1 inactivation in plasma since GLP-1 promotes insulin production when blood glucose levels are high. Sitagliptin lowers HbA1c, postprandial, and fasting glucose levels by stimulating insulin production while inhibiting the release of glucagon in a glucose-dependent way. GLP-1 has various additional effects, including slowing stomach emptying, lowering glucagon levels, and perhaps influencing appetite from the central nervous system.
- **Dosage:** Maximum 100 mg/day PO in adults and elders.
- **Drug-Drug interactions:** By blocking β-receptors in the pancreas, β-blockers prevent catecholamines release, resulting in hyperglycemia. Through activation of the α- and β-receptors, sympathomimetics and epinephrine boost hepatic glucose synthesis and glycogenolysis, inhibiting insulin secretion, resulting in hyperglycemia.
- **Significant adverse reactions:** Pancreatitis, exfoliative dermatitis, pharyngitis, headache, diarrhoea, stomach pain, nausea, vomiting, urticaria, pruritis, myalgia, joint pain, back pain, Stevens-Johnson syndrome, angioedema, heart failure, anaphylactoid reactions, rhabdomyolysis, renal failure, peripheral edema, hypoglycemia, stomatitis, oral lesions, bowel problems, bullous [354].

2.3.2.3. PK properties of Sitagliptin [176, 177]

- **Absorption:** Sitagliptin gets absorbed rapidly, with an absolute bioavailability of 87% after oral administration. In two hours, sitagliptin achieves its peak plasma concentration.
- **Volume of distribution:** In healthy subjects, the volume of distribution is 198 L.
- **Protein binding:** The fraction of sitagliptin that binds to proteins reversibly is lower (38%).
- **Metabolism:** Sitagliptin has a low metabolic rate. CYP3A4 and CYP2C8 were the main enzymes responsible for restricted metabolism. P-gp showed to have an effective efflux transport of sitagliptin.
- **Elimination:** Most sitagliptin elimination is unaltered in the urine, with metabolism being a minor route of elimination. Within one week of administration of an oral [14C] sitagliptin dosage to healthy participants, the excretion of the administered radioactivity was almost 100% via feces (13%) or urine (87%) in healthy subjects. Renal elimination, which includes active tubular secretion, mostly excreted Sitagliptin.
- **Half-life:** The half-life of sitagliptin is 12.4 hours.
- **Clearance:** Renal clearance of sitagliptin is 350 mL/min [taking 100 mg orally in healthy patients].
- **Toxicity:** There is a 34% relative risk increase for all causes of infection with an association with sitagliptin.

2.3.3. Piperine

Hans Christian Orsted, who discovered piperine in 1819, obtained it from the berries of *Piper nigrum*, the Piperaceae family's parent plant for both white and black pepper grains [178]. Black pepper is the dried ripe berry of a climbing, perennial plant prevalent in the hot, humid parts of Southern India. Pepper is said to have evolved in the mountains of India's South Western Ghats. It has now spread beyond the country of origin to Malaysia, Indonesia, Sri Lanka, China, Vietnam, Brazil, Cambodia, Guatemala, and Mexico. In India, pepper is referred to as Kali Mirch in Hindi [179]. Pepper is often used in meat processing, pickling, canning, and baking. It additionally modifies the unique flavour of cooking at the end. In the Indian medical system, black pepper is a necessary component [179]. Black and long pepper are used worldwide in various traditional systems of medicine and as household spices [180].

2.3.3.1. Physicochemical properties of Piperine

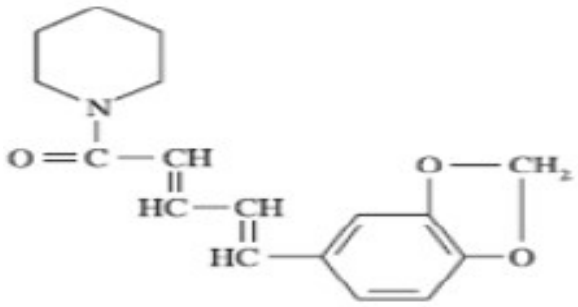


Figure 2.6. Chemical structure of Piperine [181].

Table 2.14. Physicochemical properties of Piperine [181-184].

Parameter	Properties
IUPAC Name	(2E,4E)-5-(2H-1,3-Benzodioxol-5-yl)-1-(piperidin-1-yl)penta-2,4-dien-1-one
Chemical name	1-piperoyl piperidine
Molecular formula	$C_{17}H_{19}NO_3$
Appearance	Needle-shaped or short rod-shaped light yellow or white crystalline powder
Taste	Tasteless at first, but the burning aftertaste
Molecular weight	285.34 gm/mol
Density	1.193gm/cm^3
Melting point	130^oC
Solubility	Insoluble in water but soluble in benzene and acetic acid
pKa	12.22
Stereoisomer	Isopiperine, isochavicine and chavicine

2.3.3.2. Pharmacological and therapeutic effects of Piperine

Piperine has various pharmacological effects, which might lead to great therapeutic results [185, 186]. For instance, piperine has prominently shown anti-inflammatory, hepatoprotective, antioxidant, and anti-ulcer properties in preclinical examinations [11]. Additionally, piperine prevents mice from developing lung cancer brought on by benzo(a) pyrene by reducing cell growth and protein deterioration [187]. A research study reports that piperine may affect the expression and function of the efflux transporter P-glycoprotein (P-gp). Along these lines, piperine's anticancer properties and its inhibitory effect on P-gp have piqued the curiosity of researchers looking for natural anticancer medicines that may alter multidrug resistance in chemotherapy [188]. In addition, continuing research has elucidated several added therapeutic aids of piperine in CNS and CV diseases [189]. In an animal model of Alzheimer's disorder, piperine improved neurodegeneration and spatial memory [190]. Li et al. showed the antidepressant activity in rats with piperine, where piperine enhanced the cerebrum convergence of serotonin and noradrenaline [191].

Piperine inhibits the activity of drug transporters (P-gp) as well as metabolic enzymes (CYP3A4, CYP1A2, CYP2D6, and CYP2C9), affecting the absorption and metabolic processing of co-administered medicines and resulting in piperine-drug interactions [12]. In Caco-2 cells, piperine blocked the P-gp-mediated transport of medications, including cyclosporine A and digoxin (human colon carcinoma cell lines). Research has shown that dietary piperine affects human plasma concentrations of P-gp and CYP3A4 metabolizing enzymes, particularly when oral medications are taken [192]. When piperine was administered orally to rats for 14 days at a 112g/kg dose, the intestinal P-gp increased, the hepatic P-gp decreased, and the renal P-gp remained unaffected [193]. Piperine alters the glucuronidation rate by reducing endogenous UDP-glucuronic acid concentration and suppressing transferase activity [194]. Piperine inhibits Uridine Diphosphate-Glucose Dehydrogenase (UDP-GDH) activity in the gut and liver by a non-competitive mechanism (Fig. 5) [195].

Piperine acts as a thermonutrient, and increasing thermogenesis might increase nutrient absorption. The autonomous nervous system is central to the dominant explanation of food-induced thermogenesis. The α- and β-adrenergic receptors, which are found in the gastrointestinal tract, represent the autonomous nervous system. Beta receptors, which contain cyclic adenosine 3', 5' monophosphate (cAMP), are responsible for most food or thermonutrient-induced thermogenesis. The importance of cAMP as a "second messenger" in the body's hormonal and enzymatic activities is well recognized. The requirement for fresh nutrients to maintain metabolic activities increases dramatically during thermogenesis. Independent investigations have discovered that piperine stimulates the production of catecholamines, and cAMP promotes the activation of thermogenic hormones. The thermogenic response induced by catecholamines is only short-lived [196]. Subacute piperine treatment has demonstrated significant antihyperglycemic action, but acute piperine administration increased blood glucose at large dosages [197].

2.3.3.3. PK properties of Piperine

Regardless of the route of administration, 97% of piperine gets absorbed. Piperine transport is greater in duodenal segments than ileal and jejunal segments in rats (*in-vitro*), and *in-vitro* absorption was optimum after 3 hrs of incubation, with an

absorption range of 47-64% at varying doses given to the incubation medium [198]. T_{max} and C_{max} were determined to be 2 hr and 0.983 g/ml in Wistar rats after oral administration of 20mg/kg piperine [199]. Piperine half-life in rats is 1.224 hr [199] and in humans is 13.2-15.8 hr [195]. Daniel Pushparaju Yeggon et al. reported that piperine attaches to site I (IIA) of Human serum albumin (HSA), which was confirmed by molecular dynamic and molecular docking (MD) studies on murine macrophages (RAW 264.7) cell lines [200]. Piperine clearance in rats is 8.2 µl/min/mg [199] and in humans is 10.3 µl/min/mg [201]. In rats, when piperine was administered intraperitoneally or orally, there was no elimination of piperine in urine. Still, it significantly impacted the excretion of conjugated sulphates, glucuronides, and phenols. Days 1-4 in these cases had the greatest excretion of conjugates. On the eighth day following piperine delivery, these elevated levels stabilized. The piperine has been demethylated, as shown by increased conjugated phenols.

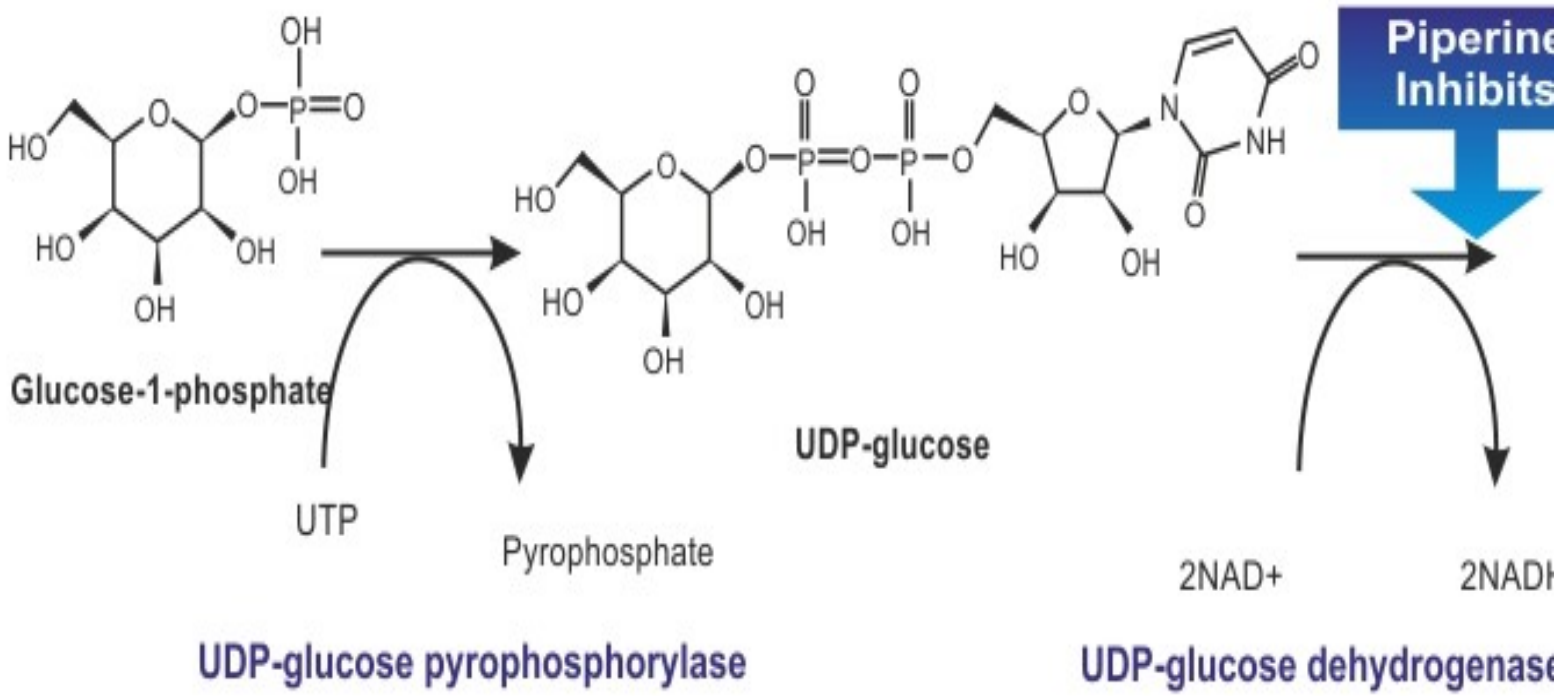


Figure 2.7. Metabolic pathway of glucuronidation showing piperine inhibition of UDP-Glucuronic acid and UDP-GDP.

2.3.3.4. PD interactions mediated by Piperine

The key data from the selected literature is summarized as follows:

1. Lala *et al*. [202] evaluated the PD interactions study of Trikatu with diclofenac sodium using a carragenin-induced rat hind paw edema model. The experimental findings observed an anti-inflammatory activity, i.e. diclofenac alone treated rats, showed a greater effect of edema inhibition than its combination with Trikatu. Hence, the study may exclude the activity elicited by the combination of diclofenac and Trikatu due to Trikatu itself, but its reason for PD interaction is unclear.
2. Veeresham *et al*. [203] investigated the effect of piperine on glimepiride in normal and diabetic rats. In PD studies, the combination of glimepiride with piperine provided significant protection against diabetes-induced alterations by showing enhanced blood glucose reduction, which explains antihyperglycemic activity.
3. Lagisetty *et al.* [204] investigated the PD interaction between piperine and gliclazide in rats and rabbits. The combination of Gliclazide with Piperine (showed 49.6% blood glucose reduction) produced greater hypoglycemic activity in normal rats when compared with alone gliclazide (showed 45.5% blood glucose reduction). The combination of Gliclazide with Piperine (showed blood glucose reduction of 68.8% in diabetic rats and 62.0% in diabetic rabbits) also produced greater antihyperglycemic activity in diabetic rats and rabbits when compared with alone gliclazide (showed blood glucose reduction of 56.4% in diabetic rats and 47.5% in diabetic rabbits). Single and multiple-dose combination of Piperine with gliclazide also produced significant changes by enhancing insulin levels and β-cell function in diabetic rats and rabbits. Here, the piperine enhanced the activity of gliclazide significantly and showed an additive effect. The processes of hypoglycemic development may involve either an increase in pancreatic insulin secretion from islet of Langerhans β-cells or an increase in pro-insulin form release.
4. Uma *et al.* [205] using male Swiss albino mice, examined the effectiveness of piperine combined with sodium valproate against seizures brought on by maximum electroshock. Mice were subjected to a 25mA current for 0.2 seconds

before experiencing electroconvulsive. Reduced malondialdehyde levels after the combined treatment of sodium valproate and piperine, indicated the combination's potential for neuroprotection. The findings suggested a positive PD interaction.

5. Zayed *et al.* [206] investigated the impact of combined administration of piperine and warfarin in Sprague-Dawley rats. The study's findings revealed considerably lower INR values after piperine co-administration at 24 hours compared to warfarin alone, which indicated that warfarin's anticoagulant effect has diminished. This study represents a potential PD drug interaction.

2.3.3.5. PK interactions mediated by Piperine

i. Pre-clinical studies

The key data from the selected literature is summarized as follows:

1. A study that investigated the effect of piperine on the PK of sodium valproate in rats reported a 14.8-fold increase in peak plasma concentration (C_{max}), a 4.6-fold increase in area under the curve (AUC), and slightly prolonged T_{max} in the presence of piperine. This study suggested the bio-enhancing effect of piperine [207].
2. Another research investigated PK interactions of piperine and rosuvastatin in rats. Peroral co-administration of piperine significantly increased intravenous exposure (AUC_{last}) of rosuvastatin by 73.5% and oral exposure (AUC_{last}) by 2.0-fold compared with the control group, while the cumulative biliary excretion of rosuvastatin significantly decreased. The study reported that piperine increases rosuvastatin's plasma exposure (AUC_{last}) and inhibits its transport to bile [208].
3. Lambert *et al.* [209] investigated the PK interaction of piperine with Epigallocatechin-3-gallate (EGCG) in mice and reported an increase in its bioavailability when co-administered with piperine. The rise in EGCG bioavailability may be because of the inhibition of glucuronidation and gastrointestinal transit.
4. Singh *et al*. [210] studied the outcome of piperine on the PKs of rifampicin and isoniazid in a rat model. After oral administration of a suspension of rifampicin, isoniazid, and piperine, observed significant alterations in PK parameters of

rifampicin and isoniazid. The study reported a significant increase in C_{max}, AUC, and bioavailability of rifampicin, while with isoniazid, there was a reduction in C_{max} and an increase in AUC. The study suggested that the decrease in the bioavailability of isoniazid could be because of piperine-induced delay in gastric emptying time.

5. Johnson *et al.* [211] investigated the effect of piperine on resveratrol PK characteristics in mice. The study found that resveratrol's bioavailability has significantly increased.
6. Liang *et al*. [212] investigated the effects of white pepper and its active ingredient, piperine, on the PKs of puerarin in rats by oral and intravenous administration.

   i) Oral administration of puerarin and piperine to rats significantly increased the C_{max} and $AUC_{0-\infty}$ of puerarin, while co-administration of white pepper decreased the oral absorption of puerarin.

   ii) Intravenous administration of puerarin and orally administered piperine did not modify the PKs of puerarin. However, following iv treatment, oral delivery of white pepper dramatically raised the AUC of puerarin.

   The study concluded that the concomitant administration of piperine or white pepper alters the PKs of puerarin. Despite being considerably different, the effects of white pepper and piperine on the PKs of puerarin in rats indicate possible diet-drug interactions.

7. Dietary piperine's impact on the PKs and PDs of pioglitazone was studied by Neerati et al. [213]. The study concluded that piperine enhanced the bioavailability of pioglitazone in normal and diabetic rabbits and thus suggested a beneficial role in the combination. The study indicated that piperine-induced metabolic inhibition of CYP 3A4 and 2C8 may be responsible for decreased metabolism of pioglitazone.
8. Singh *et al*. [214] studied the PKs of oxytetracycline administered orally after treating 7 White Leghorn hens with *Piper longum* (15 mg/kg). The study concluded that oral administration of *P. longum* alters the PK profile of oxytetracycline as the results revealed that treatment with *P. longum* significantly

increased area under curve, elimination half-life (T1/2), and mean residential time (MRT) while significantly reducing elimination rate constant (K_{el}).

9. Chen *et al*. [215] conducted a PK interaction study between magnolol and piperine in rats. Concomitant administration of piperine and magnolol resulted in a significant decrease in the AUC and C_{max} of magnolol. The study reported that co-administration of magnolol and piperine decreases the plasma concentration of either drug in rats. The study suggested that reduced bioavailability may associate with the inhibitory effect of magnolol on liver microsomal enzymes: CYP1A2 and CYP2E1.
10. Feng *et al* [216] investigated the PKs of linarin in rats after oral route administration of linarin alone and in combination with piperine to improve oral absorption of linarin. The results showed a significant increase in AUC, $C_{max,}$ and the T_{max} of linarin. The study reported that piperine significantly enhanced the oral absorption of linarin. Piperine-mediated inhibition of metabolism of linarin and P-gp mediated efflux have been held responsible for improved oral absorption.
11. Karan *et al*. [217] investigated the effect of single and multiple doses of Trikatu (an herbal preparation) on the PKs of rifampicin in rabbits. Administration of a single dose of Trikatu significantly decreased the C_{max} of rifampicin. The study suggested that Trikatu delays gastric emptying time, which reduces rifampicin absorption. Administration of multiple doses of Trikatu reduced the C_{max} and delayed the T_{max} of rifampicin, but it was not statistically significant. The study recommended that the co-administration of Trikatu with rifampicin should be avoided, as this may reduce its antibacterial efficacy.
12. Veeresham *et al*. [203] investigated the influence of piperine on glimepiride in rats. Glimepiride and piperine combination markedly increased C_{max}, AUC_{0-n}, AUC_{total}, $T_{1/2}$, and MRT, while it decreased the clearance, Vd in normal and diabetic rats as compared with the control group. The results indicated that the combination increased the bioavailability of glimepiride by inhibiting the CYP2C9 enzyme. This study suggested that piperine could be beneficial as an adjunct to glimepiride at an appropriate dose in diabetic patients.
13. Piperine's effects on the PKs and PDs of gliclazide in rats and rabbits were examined by Lagisetty et al. [204]. The study reported an additive effect for the

combination. Piperine exhibited significant hypoglycemic activity and significantly enhanced the activity of gliclazide. Piperine altered the PK parameters of gliclazide. The probable reason for the piperine-induced enhancement of gliclazide may be the inhibition of human CYP 2C9.

14. Another study investigated the influence of piperine on the oral exposure of fexofenadine in rats. Concomitant use of piperine significantly increased the AUC and bioavailability of fexofenadine, while they observed no alterations in the intravenous PKs of fexofenadine. Based on these findings, the current study suggested that piperine may increase the gastrointestinal absorption of fexofenadine, likely by inhibiting P-gp-mediated cellular efflux during intestinal absorption. The research emphasized the necessity for careful monitoring for any possible drug-diet interactions while using piperine or a piperine-containing diet in combination with fexofenadine [218].
15. In Layer birds, Patel *et al*. [219] investigated the PK interaction of piperine with gatifloxacin. The results suggested an increase in bioavailability in the combination-treated group. The study indicated that piperine inhibits the enzymes responsible for the metabolism of gatifloxacin in the liver, which enhances free drug concentration for a more prolonged duration in the body.
16. Shah *et al*. [220] studied the consequence of piperine on the PKs of macrolides (azithromycin and erythromycin) in rats. The study results revealed an increased AUC, C_{max}, and T_{max} with piperine co-administration. The study concluded that piperine significantly enhanced the bioavailability of azithromycin and erythromycin. It could be due to piperine-induced inhibition of the CYP3A4 enzyme, a drug-metabolizing enzyme of macrolides.
17. The study has reported a very low bioavailability for emodin (1,3,8-trihydroxy-6-methylanthraquinone), a potential chemopreventive agent in preclinical studies. Di *et al*. used piperine as a bio-enhancer to enhance the bioavailability of emodin. The investigated PK profiles of emodin in combination with piperine resulted that piperine significantly increased the bioavailability of emodin and possibly due to the inhibition of glucuronidation of emodin [221].
18. Athukuri *et al*. [222] performed the PK drug interaction study of piperine with domperidone in rats. The results revealed an increased C_{max}, AUC, and decreased

T_{max}, K_{el} of domperidone. Inhibition of CYP3A1 and P-gp is why piperine-induced enhanced oral bioavailability of domperidone.

19. Li *et al*. [301] conducted drug interaction studies with piperine in rats. The results expressed that piperine can potentially increase the bioavailabilities of rosuvastatin, puerarin, and docetaxel. Alteration in bioavailability is due to inhibition of CYP3A4 and P-gp activities.
20. Qiang *et al*. [223] deliberate the PK drug interaction of piperine with diltiazem in rats following its intravenous and oral administration. The study reported that pretreatment with piperine significantly decreased the bioavailability of oral diltiazem and the metabolite-parent ratio (significant reduction in desacetyldiltiazem, a major active metabolite of diltiazem). However, no significant effect resulted in the intravenous PKs of diltiazem. As diltiazem is a P-gp substrate, the study investigated the effect of piperine on the gene expression of P-gp. The study observed that pretreatment with piperine significantly enhanced the expression of intestinal P-gp in rats and increased the pregnane-X-receptor (PXR) activity in human hepatoma cells.
21. Singh *et al.* [224] investigated how piperine affected the PKs of the anti-leprosy medication dapsone in rats. The study reported an increase of 62% in peak plasma levels of dapsone in the presence of piperine. The study concluded that piperine significantly enhances the bioavailability of dapsone, and the dapsone-piperine combination may reduce dosage and side effects, particularly methemoglobinemia.
22. Hiwale *et al*. [225] investigated how piperine affected the PKs of two beta-lactam antibiotics in rats: amoxicillin trihydrate and cefotaxime sodium. The results showed that the co-administration of piperine improved the bioavailability of the antibiotics. They attributed the increased bioavailability because of piperine on microsomal metabolizing enzymes or enzymes system.
23. Janakiraman *et al*. [226] carried out the PK drug interactions study of piperine with ampicillin and norfloxacin in animal models. The study reported that co-administration of piperine improved the oral bioavailability of both antibiotics. The results suggested that piperine-induced changes in the permeability of

gastrointestinal epithelial cells and inhibition of enzymes involved in converting ampicillin to penicilloic acid may contribute to alterations in PK parameters.

24. Dama *et al*. [227] examined the effect of herbal bio-enhancer, Trikatu (a branded herbal medicine that contains piperine as an ingredient) on the PKs of pefloxacin in animals. The study's observations showed an increase in AUC, AUMC, MRT, $T_{1/2}$, and bioavailability of pefloxacin following trikatu administration.
25. Singh *et al*. [228] conducted the PK drug interactions study of piperine with metronidazole in rabbits. The plasma concentration of metronidazole increased by 57% when combined with piperine (10 mg/kg) as compared with metronidazole (20 mg/kg) alone. This study also supported the bio-enhancer effect on the bioavailability of metronidazole. The study suggested that piperine typically decreases the transepithelial electrical resistance and increases the pore size between the cells and the permeability of the intestinal milieu, which results in a higher rate and possible extent of absorption of metronidazole.
26. Singh *et al.* [229] investigated the impact of piperine on the PKs of atenolol in rats. The research demonstrated a substantial increase in atenolol's bioavailability in the presence of piperine and validated the compound's function as a bio-enhancer.
27. Boddupalli *et al*. [230] investigated the PK profile of a gastroretentive formulation of omeprazole and piperine. The concomitant administration of piperine with the gastroretentive formulation of omeprazole resulted in augmented absorption and diminished metabolism of omeprazole. The study suggested that piperine-induced membrane fluidity and efflux protein inhibition results in enhanced drug absorption.

ii. Clinical studies

1. In healthy individuals, Bano et al. [231] investigated the effect of piperine on the PK of propranolol and theophylline. The study reported an increase in AUC and C_{max} of propranolol and theophylline when administered along with piperine. The study indicated the enhancer effect of piperine on the systemic availability of oral propranolol and theophylline.

2. In individuals with uncontrolled epilepsy, Pattanaik *et al.* [232] looked into the effect of piperine on the steady-state PK of phenytoin. According to the study, piperine significantly increased phenytoin's bioavailability by increasing its absorption.
3. Bano *et al*. [233] deliberated the PK profile of piperine and phenytoin in a crossover study. Healthy participants were given either a single oral dosage of phenytoin (300 mg) or many doses of piperine (20 mg x 7 days), preceded by an oral dose of phenytoin, in research. Compared to phenytoin alone, the study found that adding piperine to phenytoin lowered absorption half-life, delayed elimination half-life, and increased AUC and concluded that the administration of multiple doses of piperine alters the PK profile of phenytoin.
4. Kasibhatta *et al*. [234] conducted a crossover, placebo-controlled study of piperine with nevirapine in healthy males aged 20-40. The study found that giving nevirapine together with piperine increased its bioavailability.
5. Bedada *et al*. [235] examined the consequence of herbal drugs containing piperine on diclofenac in healthy volunteers. The study indicated a drug-phytochemical interaction as treatment with piperine altered the PKs of diclofenac, i.e. significant rise in C_{max}, AUC, $T_{1/2}$, and a significant decrease in K_{el} was observed. The study suggested piperine-mediated inhibition of the CYP2C9 enzyme as the putative mechanism for altered PKs of diclofenac and recommended the combination therapy of diclofenac.
6. Shoba *et al*. [236] investigated the combination therapy of curcumin with piperine in healthy volunteers intending to improve the bioavailability of curcumin as piperine inhibits hepatic and intestinal glucuronidation. Compared to curcumin alone, the concomitant administration of piperine with curcumin significantly increased the bioavailability of curcumin. The study supported the bio-enhancer effect of piperine on curcumin and suggested that piperine-mediated inhibition of curcumin metabolism contributes to altered disposition or bioavailability of curcumin.
7. Bedada *et al.* [237] investigated the effects of piperine on carbamazepine's PK, a widely used antiepileptic drug, through an open-label, 2 period, sequential study conducted on 12 healthy volunteers. Piperine 20 mg o.d. was administered for 10

days during the treatment phase, while carbamazepine 200 mg o.d. was issued during the control and treatment phases. Compared to control, piperine significantly enhanced C_{max}, AUC, and $T_{1/2}$ of carbamazepine while significantly decreasing its K_{el} and apparent oral clearance. Obtained results suggested a PK interaction between piperine and carbamazepine and attributed piperine-mediated inhibition of CYP3A4 enzyme for its altered PK.

3. HYPOTHESIS, AIM & OBJECTIVES

3. 1 Hypothesis

In 1974, WHO began urging impoverished countries to supplement modern pharmacotherapy with traditional herbal treatments so to satisfy needs not addressed by conventional drugs [6]. In India, already traditional system of medicine is quite prevalent and people use different natural or herbal products in addition to allopathic medicines. Natural or herbal products are generally considered as safe but the concomitant use of herbs with drugs may lead to HDIs, which may be due to alteration in the "victim" drug's PK or (PD) caused by the herb or herbal product. Thus HDIs can affect both PK and PD of co-administered drug. Because of these HDIs, co-administration of herbal products and allopathic drugs may produce additive, synergistic, or antagonistic effects. HDIs can be a double-edged sword, posing both dangers (adverse drug effects) and advantages (by enhancing performance). Thus HDIs may enhance or reduce the activity of drug or result in drug-related toxicity as well. So it is very important to explore such putative HDIs so as to avoid harmful interactions and to exploit the beneficial ones for the management of chronic illnesses with high morbidity and mortality rates like DM.

DM is a disease in which the body does not produce enough insulin or does not respond to it correctly, resulting in abnormally high blood glucose levels [44]. Although different classes of anti-diabetic medications are available in market yet the use of herbal products as supplementary medicines is very prevalent in diabetic patients. It has been reported that 31% of diabetic patients take alternative medicine along with anti-diabetic drugs. So these patients with diabetes are more susceptible to drug interactions typically due to the use of a wide variety of drugs.

Black pepper is extensively used in a variety of ailments including DM as per traditional Indian system of medicine. In India, it is commonly used as a house hold spice and is taken by diabetic patients as an herbal remedy. One of its active phytoconstituent is piperine, which has exhibited a variety of pharmacological effects like hepatoprotective, anti-inflammatory, antioxidant, anti-ulcer and anti-diabetic effects.

Piperine has been reported to interact with various therapeutic agents thereby altering their absorption, membrane transport or metabolism. It has been reported that piperine can inhibit human CYP3A4, CYP1A2, CYP2D6, CYP2C9 enzymes, and P-gp transporters, which are crucial for metabolism and transport of xenobiotics/ metabolites respectively. As Repaglinide and Sitagliptin are mainly metabolized by CYP3A4 and CYP2C8 enzymes, their interaction with piperine is very likely. It acts as a bioenhancer and enhances bioavailability of other drugs. It is one of the primary active components of the diabetes patients' medications sugurd capsules and diabecon tablets. As a result, piperine may be used concurrently with oral hypoglycemic medications.

Earlier studies have reported PK/PD interactions of piperine with different antidiabetic drugs. However, there is no report/evidence regarding the influence of piperine on the PK/PD of repaglinide and sitagliptin which is critical in clinical practice so as to provide rational therapy. So the aim of this study was to investigate the influence of piperine on PD & PK of selected antidiabetic drugs (repaglinide and sitagliptin) in animal models based on the hypothesized possibilities of interaction between them.

3.2 Aim

To investigate PK and PD interactions of repaglinide and sitagliptin with piperine in animal models.

3.3. Objectives

- To investigate the pharmacodynamic interactions of selected anti-diabetic drugs (Repaglinide & Sitagliptin) with piperine by measuring the blood glucose and insulin levels in **normal and diabetic Wistar rats.**
- To investigate the pharmacodynamic interactions of selected anti-diabetic drugs with piperine by measuring the blood glucose and insulin levels in **normal and diabetic rabbits.**
- To investigate the pharmacokinetic interactions of selected anti-diabetic drugs with piperine in **normal rabbits** by measuring the pharmacokinetic parameters.

- To investigate the pharmacokinetic interactions of selected anti-diabetic drugs with piperine in **diabetic rabbits** by measuring the pharmacokinetic parameters.

4. MATERIALS AND METHODS

4.1. Materials

4.1.1. Biological fluids, chemicals and drugs

Table 4.1. Information about materials and experimental drugs

Biological fluids	**Provider**	**Purpose**
Blood	Vuppala Venkaiah Memorial Blood Bank	Plasma is extracted from the human blood to perform bioanalytical studies.
Chemicals	**Manufacturer/Provider**	**Purpose**
Acetonitrile	Standard Reagents Pvt Ltd, Hyderabad, India	These chemicals were used for the HPLC method development and validation study of Repaglinide and Sitagliptin
Triethanolamine	Vestro Solvents Pvt Ltd, Hyderabad, India	
Ammonium formate	Vishnupriya Chemicals Private Limited, Hyderabad, India	
Dichloromethane	Balaji Formulations Pvt Ltd, Hyderabad, India	
Diethyl ether	Standard Reagents Pvt Ltd, Hyderabad, India	
Diazepam	RL Fine Chem, Bengaluru, India.	
Deionized distilled water	UCC fine Chem, Hyderabad, India.	
Drugs	**Manufacturer/Provider**	**Purpose**
Piperine	Herbochem Laboratories	These drugs were administered to rats and rabbits as per methodology.
Repaglinide	Aurobindo Pharma Ltd, Hyderabad, India.	
Sitagliptin	Dr. Reddy's Laboratories, Hyderabad, India.	
Rosiglitazone	SMS Pharmaceuticals Ltd,	

	Hyderabad, India.	
Streptozotocin (STZ)	Sisco Research Laboratories Pvt. Ltd, warehouse, Hyderabad, India.	

4.1.2. Dose selection

Doses for rats and rabbits were selected on the basis of existing literature [243-245]. Antidiabetic drugs (repaglinide and sitagliptin) were dissolved in a few drops of 0.1N NaOH and then volume was made up with distilled water [244, 245]. Piperine solution was prepared in 2% gum acacia [243].

4.1.3. Experimental animals

Drug interactions are typically studied in animal models (rodents and non-rodents). The normal rats were used to assess drug interactions while the diabetic rat model was used to validate the response in the pathological condition [241, 242]. Rabbits were used to establish and confirm the interaction findings in another distinct species [242]. Table 4.2 gives the details of the animals.

Table 4.2. Details of experimental animals used in the study

Details	Wistar rats	Rabbits
Provider	Muppandal Genomics & Immunologicals Pvt. Ltd.	Muppandal Genomics & Immunologicals Pvt. Ltd.
Sex	Both male and female	Both male and female
Age	7-8 weeks	3-5 months
Weight	200-300 grams	2 - 2.5 kg
Diet	Commercial pellet diet	Uncooked Vegetable diet

The animals (table 16) were housed in standard laboratory conditions, with a 12:12 light/dark cycle with an ambient temperature of 25 ± 20°C and relative humidity of 50 ± 15%. Commercial pellet (Rayan's Biotechnologies Pvt Ltd., Hyderabad, India) diet and uncooked vegetable diet with water ad libitum was provided to rats and rabbits respectively. The animals were fasted for 18 hours before the experiment. The experimental studies were carried as per the "Committee for the

Purpose of Control and Supervision of Experiments on Animals" (CPCSEA) guidelines. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Guru Nanak Institute of Pharmacy, Ranga Reddy District, Telangana (IAEC Approval No: 04/GNIP/CPCSEA/IAEC/2019 for rabbits and 05/GNIP/CPCSEA/IAEC/2019 for rats).

4.2. Experimental study design

Experimental studies were conducted in four stages. The design of the study is as follows:

Stage 1: PD interactions in normal rats

Stage 2: PD interactions in diabetic rats

Stage 3: PD and PK interactions in normal rabbits

Stage 4: PD and PK interactions in diabetic rabbits

4.2.1. Study of PD interactions in normal rats (Stage 1): Single Dose Treatment (SDT) & Multiple Dose Treatment (MDT)

Adult Wistar rats of either sex were divided into following six groups and each group comprised of 6 animals:

Group I: Normal rats treated with Vehicle (2% w/v CMC, p.o.)

Group II: Normal rats treated with Piperine (20mg/kg, p.o.)

Group III: Normal rats treated with Repaglinide (0.5mg/kg, p.o.)

Group-IV: Normal rats treated with Piperine (20mg/kg, p.o.) and Repaglinide (0.5mg/kg, p.o.)

Group-V: Normal rats treated with Sitagliptin (10 mg/kg, p.o.)

Group-VI: Normal rats treated with Piperine (20mg/kg, p.o.) and Sitagliptin (10 mg/kg, p.o.)

For the PD study, these groups were treated for 21 days and blood samples were collected from each animal at predetermined intervals of 0^{th} day, 1^{st} day, 3^{rd} day, 7^{th} day, 14^{th} day, and 21^{st} day at 1^{st} hr for repaglinide study and 3^{rd} hr for sitagliptin study. They were used to measure glucose and insulin levels by Glucose Oxidase (GOD)-Peroxidase (POD) [246] and Enzyme-Linked Immunosorbent Assay (ELISA) method respectively [247].

4.2.1.1. Collection of blood samples from rats

Blood (<1 mL) was drawn from each rat's retro-orbital plexus at predetermined intervals. It is the best method if small amounts (< 1 mL) of blood samples are required [248]. To rupture the fragile venous capillaries of the ophthalmic venous plexus, a tiny capillary was carefully inserted in the inner angle of the eye, and then slipped beneath the eyeball at a 45-degree angle and across the bone socket. The passage was about 10 mm. The capillary's tip was gently retracted, and blood collected in the orbital cavity flowed out of the capillary and into a micro-centrifuge tube [248]. After collecting the desired volume, the capillary was removed with the simultaneous release of pressure by the forefinger and thumb. Any residual blood droplet around the eyeball was wiped off by an absorbent cotton swab. In this study, un-anesthetized animals were used because anesthesia causes hyperglycemia by various mechanisms.

4.2.1.2. Handling of blood samples

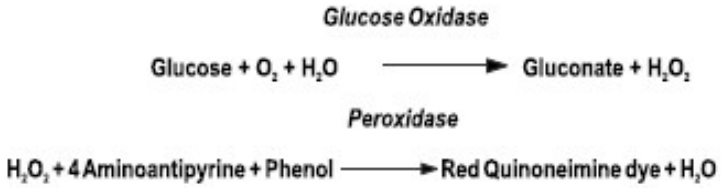
The collected blood samples were mixed with 10% Ethylenediamine Tetraacetic Acid (EDTA) solution (1 g of EDTA in 10 ml of distilled H_2O) and vortex mixed (10µl EDTA in 1ml of blood). After that, blood samples were centrifuged for 15 min at 2-8^{o}C at 2000-3000 rpm. Plasma was extracted (translucent upper layer). Collected plasma was transferred into a previously labelled tube for further use.

4.2.1.3. Estimation of glucose levels

Glucose levels were determined using the GOD-POD technique, as directed in the kit handbook.

i. Principle

Under the effect of glucose oxidase, glucose is transformed to gluconate and hydrogen peroxide. Peroxidase catalysed the reaction of hydrogen peroxide, phenol, and 4-aminoantipyrine, resulting in a red quinoneimine colouring complex. The produced colour intensity indicates the glucose quantity in a blood sample.



ii. Reagents

Table 4.3: Description of GOD-POD test reagents

Reagent L1	Glucose reagent	Glucose oxidase, Peroxidase, Buffer, 4-aminoantipyrine, Stabilizers
Reagent S	Glucose standard (100mg/dl)	Dextrose, Benzoic acid

iii. Procedure

Clean, dry test tubes were labelled with the letters B (Blank), S (Standard), and T (Test). The sequence of addition of reagents and plasma is stated in table 4.4.

Table 4.4: Working table for GOD-POD procedure

Addition sequence	**B (ml)**	**S (ml)**	**T (ml)**
Glucose Reagent	1.0	1.0	1.0
Distilled Water	0.01	-	-
Glucose Standard	-	0.01	-
Sample (Plasma)	-	-	0.01

After thoroughly mixing the ingredients, incubation was done for 10 minutes at 37°C. Within 60 minutes, the study compared the Standard Sample (Abs.S) and

Test Sample (Abs.T) with the absorbance of the blank. The study used the following formula for blood glucose level estimation:

$$\text{Total Glucose in mg/dl} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 100$$

4.2.1.4. Estimation of insulin levels

Insulin levels were determined by the ELISA method as per instructions in the kit manual.

i. Principle

The plate was pre-coated with rat insulin antibody in this ELISA method. Insulin in the sample was added to all wells, where it interacts with antibodies. The sample is added with the biotinylated rat insulin antibody, which adheres to the insulin. After that, Streptavidin-HRP, which interacts with the biotinylated insulin antibody, was added. All free Streptavidin-HRP has splashed away during the rinsing step after incubation. The substrate solution was added, and the hue formed to the quantity of rat insulin present. An acid-stop solution was used to stop the process, and the absorbance was assessed at 450 nm.

ii. Preparation of standard for rat insulin ELISA

To make a 24mU/L standard solution, reconstitute 120µl standard solution (48mU/L) with 120µl standard diluent. The standard was allowed for 15 minutes with moderate swirl before making dilutions. Identical standards were made by mixing the standard stock solution (24mU/L) with standard diluent to obtain 12mU/L, 6mU/L, 3mU/L, and 1.5mU/L solutions (table 4.5 and figure 4.1). The zero standard (0mU/L) was standard diluent. Any leftover solution was frozen at -20°C for future Use. The following are the proposed dilutions of standard solutions:

Table 4.5: Serial dilution of rat insulin standard concentration

24mU/L	Standard 5	Original Standard 120µl + Standard diluent 120µl
12mU/L	Standard 4	Standard 5 120µl + Standard diluent 120µl
6mU/L	Standard 3	Standard 4 120µl + Standard diluent 120µl
3mU/L	Standard 2	Standard 3 120µl + Standard diluent 120µl
1.5mU/L	Standard 1	Standard 2 120µl + Standard diluent 120µl

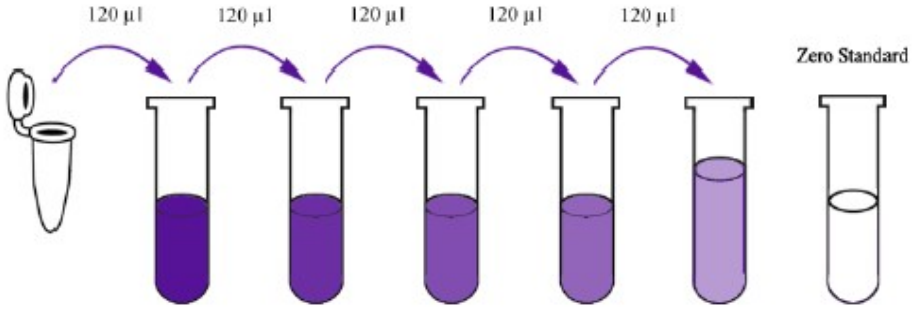


Figure 4.1. Serial dilution of rat insulin standard concentration

Table 4.6: Standard concentrations of rat insulin

Standard Concentration	Standard-5	Standard-4	Standard-3	Standard-2	Standard-1
48mU/L	24mU/L	12mU/L	6mU/L	3mU/L	1.5mU/L

iii. Assay procedure for ELISA

1. Kit instructions were followed for preparing all reagents, standard solutions (tables 4.5 & 4.6), and samples. Each reagent was fetched to ambient temperature before use. At ambient temperature, the test was carried out.

2. The strips were placed in the appropriate frames. Strips that aren't in use were stored at 2-8°C.

3. In the standard well, added 50µl of standard. Note: The antibody should not pour into the standard well since the standard solution already includes a biotinylated antibody.

4. 40µl of the sample was added in sample wells, followed by 10µl anti-insulin antibody, 50µl streptavidin-HRP in sample wells, and 50µl streptavidin-HRP in

standard wells (Not blank control well). The reaction mixture was mixed thoroughly and incubated at 37°C for 60 minutes.

5. The plate was cleansed five times using a wash buffer. For each wash, wells were submerged in at least 0.35mL of wash buffer for 0.5 to 1 minute. All wells were aspirated, and wash buffer was used to wash five times, overfilling the wells to enable automated washing. Plate was blotted with paper towels.
6. In each well, 50µl of substrate solution 'A' was added and 50µl of substrate solution 'B' was added. The plate was incubated in the dark at 37°C for 10 minutes before sealing it.
7. The blue hue immediately became yellow when 50 µl of Stop Solution was transferred to every well.
8. The optical density (OD) of every well was evaluated by operating a microplate reader at 450 nm for 10 minutes after adding the stop solution.

iv. Calculation of result

Using computer-based curve-fitting techniques, the standard curve was constructed by graphing the mean OD for every standard (Y-axis) against the concentration (X-axis).

4.2.2. Study of PD interactions in diabetic rats (Stage 2): SDT & MDT

4.2.2.1. Induction of diabetes in rats

Overnight fasted rats were administered nicotinamide (NA) 210 mg/kg i.p (in phosphate buffer solution) [249]. After 15 minutes, STZ 55 mg/kg in citrate buffer (0.1M, pH 4.5) was injected intraperitoneally to induce type-2 DM [250, 251]. Elevated blood glucose levels determined the induction of hyperglycemia. The blood glucose levels of all animals that received STZ-NA exhibited a triphasic response with hyperglycemia at 1 hr followed by hypoglycemia lasting for 6 hr and finally stable hyperglycemia by 24-48 hr [252]. The study included animals with fasting blood glucose levels more than 250mg/dl. Diabetic rats of either sex were divided into the following six groups, and each group comprised 6 animals:

Group I: Diabetic rats treated with Vehicle (2% w/v CMC, p.o.)

Group II: Diabetic rats treated with Piperine (20mg/kg, p.o.)

Group III: Diabetic rats treated with Repaglinide (0.5mg/kg, p.o.)

Group IV: Diabetic rats treated with Piperine (20mg/kg, p.o,) and Repaglinide (0.5mg/kg, p.o.)

Group V: Diabetic rats treated with Sitagliptin (10 mg/kg, p.o.)

Group VI: Diabetic rats treated with Piperine (20mg/kg, p.o.) and Sitagliptin (10 mg/kg, p.o.)

These groups were treated for 21 days and blood samples were collected from each animal at predetermined intervals of 0^{th} day, 1^{st} day, 3^{rd} day, 7^{th} day, 14^{th} day, and 21^{st} day at 1^{st} hr for repaglinide study and 3^{rd} hr for sitagliptin study. They were used to measure glucose and insulin levels by GOD-POD [246] and ELISA method respectively [247].

4.2.3. Study of PD and PK interactions in normal rabbits (Stage 3): SDT & MDT

Adult rabbits of either sex were divided into the following six groups and each group comprised of 4 animals:

Group I: Normal rabbits treated with Vehicle (2% w/v CMC, p.o.)

Group II: Normal rabbits treated with Piperine (20mg/kg, p.o.)

Group III: Normal rabbits treated with Repaglinide (0.3mg/kg, p.o.) [253]

Group IV: Normal rabbits treated with Piperine (20mg/kg, p.o.) [243] and Repaglinide (0.3mg/kg, p.o.) [254]

Group V: Normal rabbits treated with Sitagliptin (7 mg/kg, p.o.)

Group VI: Normal rabbits treated with Piperine (20mg/kg, p.o.) and Sitagliptin (7 mg/kg, p.o.) [254]

As mentioned above, these groups were treated for 21 days and blood samples were collected from each animal at predetermined intervals of 0^{th} day, 1^{st} day, 3^{rd} day, 7^{th} day, 14^{th} day, and 21^{st} day.

4.2.3.1. Collection of blood samples from rabbits

Blood samples (≈2 ml) were acquired from each rabbit's marginal ear vein at predetermined intervals. Rabbits were kept in a cage. Blood vessels were inflated by massaging the ears with cotton gauze, and the left ear was shaved for ease. The blood was taken in micro-centrifuge tubes, subsequently puncturing the inflated blood vessel of the left small ear vein with a sharp needle syringe [255]. After drawing the blood, the collection site was covered with clean, sterile cotton and finger compression was done to stop the flow.

i. Study of glucose and insulin levels in normal rabbits

Blood samples were used to measure glucose and insulin levels by GOD-POD [246] and ELISA methods, respectively [247].

ii. Study of PK parameters in normal rabbits

Blood samples were collected from each animal at predetermined intervals i.e. for Repaglinide 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 hr and for Sitagliptin 0, 1, 2, 3, 4, 8, 16, 24 hr on 1^{st} and 21^{st} day. These blood samples were subjected to HPLC analysis to estimate Repaglinide and Sitagliptin in the rabbits' plasma.

4.2.4. Study of PD and PK interactions in diabetic rabbits (Stage 4): SDT & MDT

4.2.4.1. Induction of diabetes in rabbits

Overnight fasted rabbits were administered NA 1000 mg/kg i.p (in phosphate buffer solution) [250]. After 15 minutes, STZ 65 mg/kg in citrate buffer was injected intraperitoneally to induce type 2 DM [251]. Elevated blood glucose levels determined the induction of hyperglycemia. The blood glucose levels of all animals that received STZ-NA exhibited a triphasic response with hyperglycemia at 1 hr

followed by hypoglycemia lasting for 6 hr and finally stable hyperglycemia by 24-48 hr [252]. The study included animals with fasting blood glucose levels more than 250mg/dl. Diabetic rabbits of either sex were divided into the following six groups, and each group comprised of 4 animals:

Group-I: Diabetic rabbits treated with Vehicle (2% w/v CMC, p.o.)

Group-II: Diabetic rabbits treated with Piperine (20mg/kg, p.o.) [243]

Group-III: Diabetic rabbits treated with Repaglinide (0.3mg/kg, p.o.) [253]

Group-IV: Diabetic rabbits treated with Piperine (20mg/kg, p.o.) and Repaglinide (0.3mg/kg, p.o.)

Group-V: Diabetic rabbits treated with Sitagliptin (7 mg/kg, p.o.) [254]

Group-VI: Diabetic rabbits treated with Piperine (20mg/kg/po) and Sitagliptin (7 mg/kg, p.o.) [254]

i. Study of glucose and insulin levels in diabetic rabbits

All groups were given treatment for 21 days and blood samples were taken (fasted for 14 h) from each animal at specified intervals of the 0^{th}, 1^{st}, 3^{rd}, 7^{th}, 14^{th}, and 21^{st} days. Then glucose and insulin levels were measured as discussed earlier [247].

ii. Study of PK in diabetic rabbits

Blood samples were collected from each animal at predetermined intervals i.e. for Repaglinide 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 hr and for Sitagliptin 0, 1, 2, 3, 4, 8, 16, 24 hr on 1^{st} and 21^{st} day. These blood samples were subjected to HPLC analysis to estimate Repaglinide and Sitagliptin in the rabbits' plasma.

4.2.5. Non-compartmental PK analysis

Plasma concentration vs. time data for Repaglinide and Sitagliptin were subjected to non-compartmental analysis using WinNonlin (version 5.2.1) software to estimate PK parameters (table 4.7).

4.2.6. Statistical analysis

Data was shown as mean ± SD. $p<0.05$ was considered significant. One-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Test were used to analyze PD findings and Student's paired t-Test was used to assess the PK results.

Table 4.7: PK parameters by Non compartmental analysis using WinNonlin Software

Parameter	**Description/Formula**
Time of peak concentration	T_{max} = Time of maximum observe
Peak concentration	C_{max} = Maximum observed conce
Elimination rate constant	K_{el} = Estimated by linear regressi
Area under curve	AUC = Determined via the linear
The area under curve from zero to last measurable concentration	AUC_{0-t} = Area under curve from t last measurable concentration
The area under curve from zero to infinity	$AUC_{0-\infty} = AUC_{0-t} + C_{last\ obs} / K_{el}$
Area under the first moment curve from zero to the last measurable concentration	$AUMC_{0-t}$ = Area under the momer time) to the last measurable conce
Area under the first moment curve from zero to infinity	$AUMC_{0-\infty} = AUMC_{0-t} + (T_{last} \cdot C_{last}$

5. BIO-ANALYTICAL METHOD DEVELOPMENT FOR REPAGLINIDE ESTIMATION IN RABBIT PLASMA

5.1. Optimal chromatographic conditions of Repaglinide

- **Mobile phase:** Acetonitrile : Ammonium formate at pH 3.8
  Ratio: 50:50
- **HPLC used:**

  RP18 HPLC system Symmetry shield (for water-soluble)
  With 2998 Photo Diode Areditector, quaternary pump with the software of empower 3.
  C-18 HS Column (250nm x 4.6mm x5 µm)
- **Flow rate**: 1 ml/min
- **Internal Standard used:** Diazepam
- **Retention time of Repaglinide:** 4.48 min
- **Retention time of Diazepam:** 6.59 min

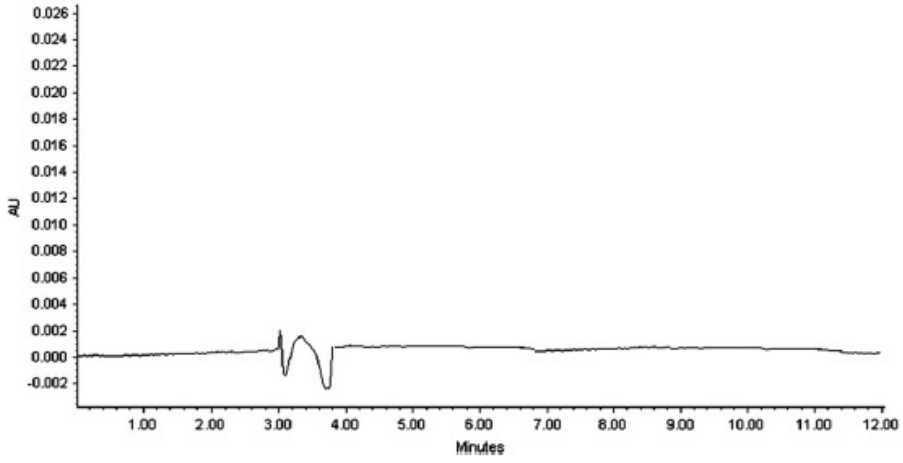


Figure 5.1. ***Blank plasma sample chromatogram***

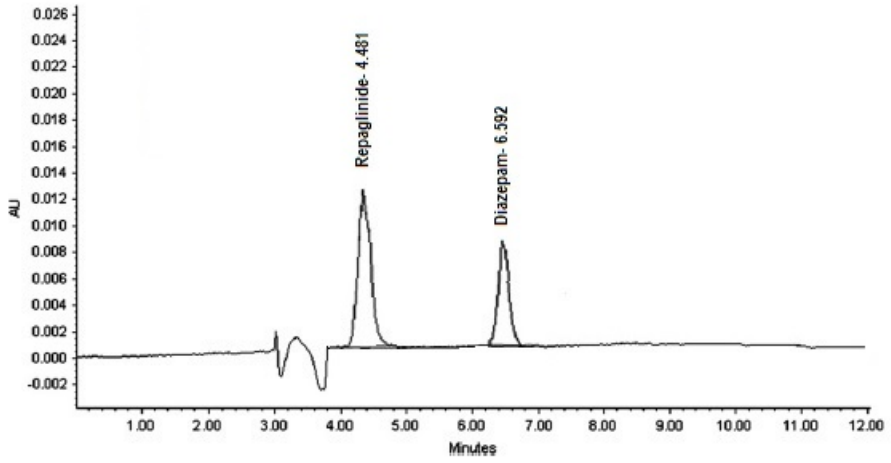


Figure 5.2. ***Repaglinide in spiked human plasma with Internal Standard of optimized chromatogram***

5.2. Preparation of Repaglinide stock and working solution

For Repaglinide 1000 µg/ml, Repaglinide 100 mg was dissolved in acetonitrile 10 ml, and the volume was diluted to 100 ml using the mobile phase. For 100 µg/ml, 10 ml of the above solution was withdrawn, and the volume was diluted up to 100 ml. Now, this was a stock solution. This stock solution yielded workable solutions with concentrations ranging from 1, 2, 4, 8, 10, and 20 µg/ml.

5.3. Calibration curve and quality control sample preparation

To produce different calibration curve standards in the range of 10, 20, 40, 80, 100, 200, and 300 ng/ml, aliquants of blank human plasma (4.95 ml) were spiked with aliquants of prepared working solutions (0.05 ml) (of 1, 2, 4, 8, 10 and 20 µg/ml). Similarly, multiple quality control samples were produced from the working solutions. Tables 5.1 and 5.2 provide further information.

Table 5.1. Preparation of calibration curve standard for Repaglinide

Working solution utilized (µg/ml)	Aliquant volume withdrawn (ml)	Human plasma volume added (ml)	Final volume (ml)	Achieved final concentration (ng/ml)
1	0.05	4.95	5	10
2	0.05	4.95	5	20
4	0.05	4.95	5	40
8	0.05	4.95	5	80
10	0.05	4.95	5	100
20	0.05	4.95	5	200
30	0.05	4.95	5	300

Table 5.2. Preparation of quality control standard samples of Repaglinide

Working solution utilized (µg/ml)	Aliquant volume withdrawn (ml)	Human plasma volume added (ml)	Final volume (ml)	Achieved final concentration (ng/ml)	Quality control Level
1	0.05	4.95	5	10	LLOQ
4	0.05	4.95	5	40	LQC
10	0.05	4.95	5	100	MQC
20	0.05	4.95	5	200	HQC
30	0.05	4.95	5	300	ULOQ

ULOQ- Upper limit of quantification, HQC- Higher quality control, MQC- Middle-quality control, LQC- Lower quality control, LLOQ- Lower Limit of Quantification

5.4. Sample preparation for the analysis by HPLC

The liquid-liquid extraction method was used to produce the sample. Six 15 ml stoppered test tubes were filled with an aliquant of spiking human plasma (1 ml) and 50 ml of internal standard working solution (10 g/ml) and swirled for 1 minute. 5 ml dichloromethane:diethyl ether (4:6, v/v) was added to this solution. The constituents of tubes were blended in a slope position at 100 strokes per minute on a reciprocating shaker for 30 minutes and centrifuged for 10 minutes at 3000 rpm to isolate the phases. Under a nitrogen stream, the supernatant layer (4 ml) was moved

to another tube and permitted to evaporate for dryness. Afterward, the residue was mixed with 250 mL of mobile phase and examined chromatographically.

The same procedure was followed for the sample preparation with rabbit plasma for HPLC analysis to estimate the plasma drug concentrations of repaglinide.

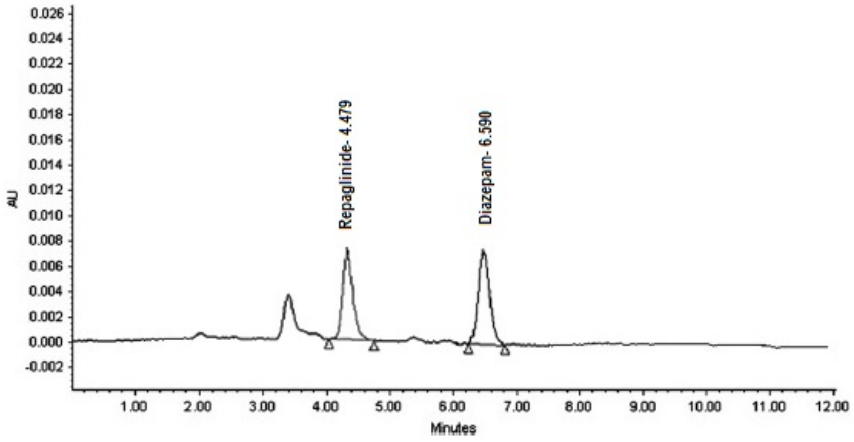


Figure 5.3. ***Chromatogram of Repaglinide with Internal Standard at LQC level***

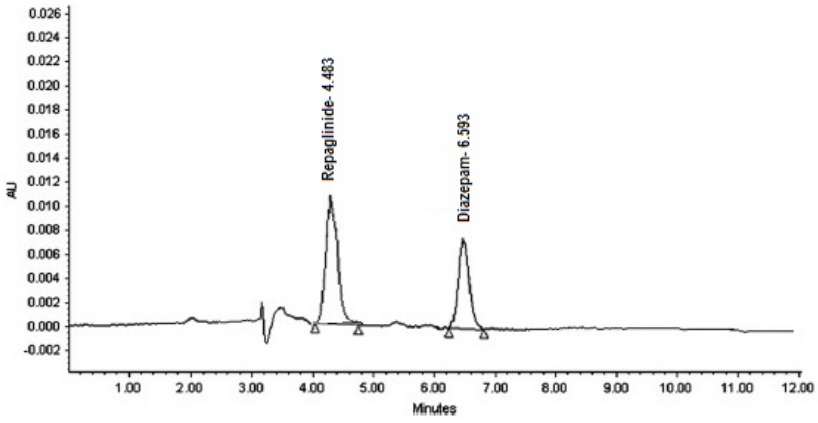


Figure 5.4. ***Chromatograms of Repaglinide with Internal Standard at MQC level***

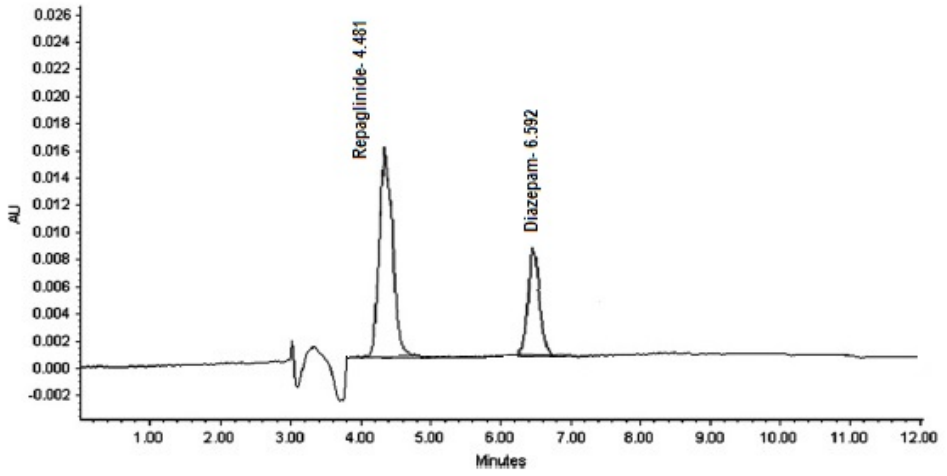


Figure 5.5. ***Chromatogram of Repaglinide with the Internal Standard at the HQC level***

5.5. Linearity results of Repaglinide

The developed method's linearity was validated in 10-300 ng/ml concentrations. Human plasma was used to produce and process the seven calibration samples. The calibration curve was drawn using a regression calculation with a weighting factor of 1/(concentration ratio)^2 of the medication to internal standard strength to get the proper tally for the concentration/response relation. Linearity acceptability requirement, the r^2 (coefficient of correlation) should be less than 0.98. Table 5.3 and Figure 5.6 show the results.

Table 5.3. Area ratios as of calibration experimentations of Repaglinide

Level of Calibration Standards	Drug Quantity (ng/ml)	Ratio of Area (n=6, Mean ± SD)
CC1	10	0.17 ± 0.02
CC2	20	0.39 ± 0.02
CC3	40	0.79 ± 0.01
CC4	80	1.56 ± 0.02
CC5	100	1.96 ± 0.08
CC6	200	3.90 ± 0.34
CC7	300	5.30± 0.02

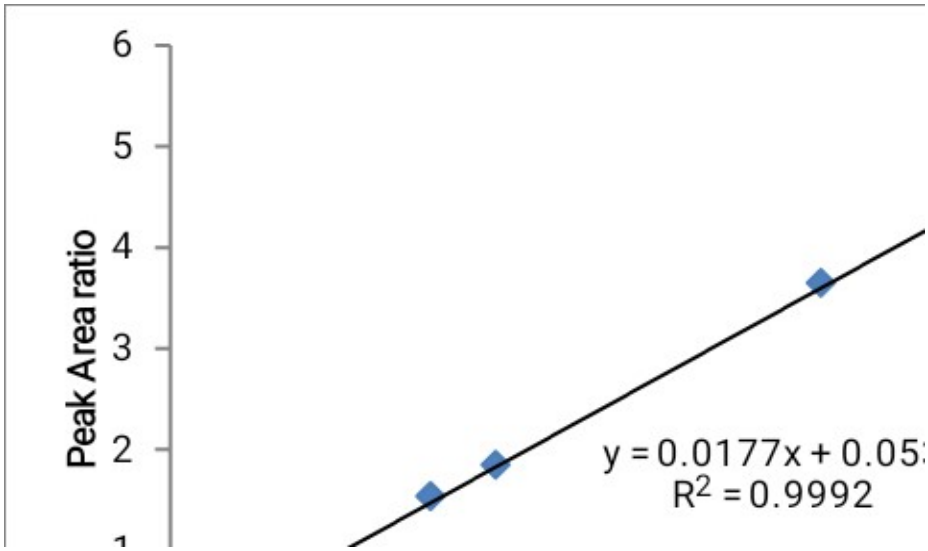


Figure 5.6. Linearity graph of Repaglinide

5.6. Results of accuracy plus precision of Repaglinide

To evaluate intra- and inter-day precision and accuracy, many quality control samples at HQC, MQC, LQC and LLOQ were utilised in six repetitions. The concentrations at HQC, MQC, LQC and LLOQ were determined, followed by standard deviation, percent CV (for precision), and percent nominal (for accuracy) for each duplicate.

The acceptability criterion for accuracy (% nominal) and precision (% CV) is ±15%, and only for the LLOQ sample is ±20%. Tables 5.4 to 5.8 show the Intraday and Interday outcomes.

Table 5.4. Day 1 Interday accuracy and precision of Repaglinide

Day 1	LOQ		MQC		HQC	
	40 ng/ml	Accuracy %	100 ng/ml	Accuracy %	200 ng/ml	Accuracy %
1	36.22	90.55	96.23	96.23	195.33	90.55
2	37.55	92.45	94.06	94.06	196.32	91.75
3	38.21	93.44	96.23	96.23	195.26	91.53
4	37.95	92.67	96.61	96.61	194.55	89.75
5	37.5	92.86	97.06	97.06	195.78	91.7
6	39.87	99.58	96.75	96.75	197.99	95.67
Mean	**37.2**		**95.66**		**195.53**	
SD	**2.123**		**21.53**		**1.088**	
%CV	**1.29**		**2.05**		**1.53**	
% Nominal	**98.56**		**96.59**		**94.94**	
N	**6**		**6**		**6**	

Table 5.5. Day 2 Interday accuracy and precision of Repaglinide

Day 2	LOQ		MQC		HQC	
	40 ng/ml	Accuracy %	100 ng/ml	Accuracy %	200 ng/ml	Accuracy %
1	36.22	90.55	95.67	95.67	194.53	96.53
2	35.55	92.45	96.58	96.58	196.39	97.2
3	37.01	93.65	98.45	98.45	197.56	98.34
4	37.95	92.67	97.46	97.46	191.25	90.55
5	36.4	92.22	97.32	97.32	197.23	97.06
6	39.87	99.56	95.71	95.71	195.92	96.86
Mean	**37.2**		**97.06**		**194.63**	
SD	**2.065**		**2.64**		**42.65**	
%CV	**1.65**		**1.53**		**2.1**	
% Nominal	**97.56**		**96.75**		**95.11**	
N	**6**		**6**		**6**	

Table 5.6. Day 3 Interday accuracy and precision of Repaglinide

Day 3	LOQ		MQC		HQC	
	40 ng/ml	Accuracy %	100 ng/ml	Accuracy %	200 ng/ml	Accuracy %
1	37.56	93.64	95.86	95.86	193..34	96.56
2	35.46	91.53	97.59	97.59	194.37	93.45
3	36.45	94.7	96.23	96.23	195.76	97.65
4	35.26	91.89	99.03	99.03	197.68	98.53
5	36.86	93.5	97.56	97.56	195.78	98.67
6	38.56	94.86	98.73	98.73	197.37	97.53
Mean	**36.86**		**95.86**		**196.73**	
SD	**2.61**		**2.86**		**4.53**	
%CV	**1.06**		**1.53**		**2.32**	
% Nominal	**96.73**		**97.49**		**95.63**	
N	**6**		**6**		**6**	

Table 5.7. Day 4 Interday accuracy and precision of Repaglinide

Day 4	LOQ		MQC		HQC	
	40 ng/ml	Accuracy %	100 ng/ml	Accuracy %	200 ng/ml	Accuracy %
1	36.02	94.64	97.84	97.84	189..34	90.43
2	37.53	96.53	99.35	99.35	193.67	96.53
3	38.46	97.7	93.24	93.24	195.76	97.56
4	37.35	96.34	99.37	99.37	198.73	98.7
5	37.4	94.39	99.64	99.64	197.53	97.53
6	39.61	95.79	97.73	97.73	196.73	96.73
Mean	**37.53**		**95.86**		**192.73**	
SD	**2.06**		**2.86**		**6.5**	
%CV	**1.43**		**1.53**		**2.53**	
% Nominal	**97.06**		**97.49**		**94.39**	
N	**6**		**6**		**6**	

Table 5.8. Intraday accuracy and precision of Repaglinide

SL No	LOQ		MQC		HQC	
	40 ng/ml	Accuracy %	100 ng/ml	Accuracy %	200 ng/ml	Accuracy %
1	38.56	96.4	96.53	96.53	194..34	97.56
2	37.90	95.64	94.23	94.23	195.37	93.63
3	37.02	95.06	93.56	93.56	192.76	97.33
4	36.93	94.83	96.64	96.64	195.68	96.51
5	38.56	97.68	95.69	95.69	194.78	95.07
6	38.65	98.65	97.43	97.43	191.37	92.03
Mean	**37.44**		**95.73**		**194.93**	
SD	**1.52**		**2.51**		**3.64**	
%CV	**1.31**		**2.01**		**2.36**	
% Nominal	**96.38**		**95.53**		**96.61**	
N	**6**		**6**		**6**	

5.7. Results of selectivity study of Repaglinide

Analyte selectivity was assessed to identify internal standard interference during analyte retention. Six blank matrix internal-standard copies were injected, and if an analyte area was identified, that was rivalled with the analyte's mean area obtained with injected LLOQ strength. Internal Standard selectivity for six blank matrix duplicates with drugs injected at the ULOQ-level was also assessed. The resulting internal-area standard was then compared with the mean internal-area standard obtained at the LLOQ level. Tables 5.9 to 5.11 show the results.

Table 5.9. Crest area of Repaglinide and area of Internal Standard

Sample Id	Repaglinide Crest Area	Internal Standard Crest Area
LLOQ-1 Selectivity	11506	12028
LLOQ-2 Selectivity	11253	12382
LLOQ-3 Selectivity	11352	12286
LLOQ-4 Selectivity	11012	12539
LLOQ-5 Selectivity	12058	12846
LLOQ-6 Selectivity	11135	12039
Mean	**11143.1**	**12684.34**
SD	**98.33**	**241.33**
%CV	**0.86**	**0.26**

Table 5.10. Analyte selectivity study of Repaglinide

Sample Id	Repaglinide Crest Area	Internal Standard Crest Area
(Internal Standard + Blank)-1	0	12203
(Internal Standard + Blank)-2	0	12183
(Internal Standard + Blank)-3	0	12938
(Internal Standard + Blank)-4	0	12349
(Internal Standard + Blank)-5	0	12013
(Internal Standard + Blank)-6	0	12886
Repaglinide (analyte)with an Internal Standard mean response	**0**	
Repaglinide (analyte) in LLOQ selectivity mean response	**11143.1**	
% of interference at analyte retention time with Internal Standard	**0**	

Table 5.11. HPLC study of Repaglinide Internal Standard selectivity

Sample Id	Repaglinide Crest Area	Internal Standard Crest Area
ULOQ1 Repaglinide	361049	0
ULOQ2 Repaglinide	358686	0
ULOQ3 Repaglinide	352591	0
ULOQ4 Repaglinide	348843	0
ULOQ5 Repaglinide	356512	0
ULOQ6 Repaglinide	359859	0
Internal standard with repaglinide (analytes) means response.		**0.00**
Internal Standard in LLOQ selectivity means response		**12684.34**
% of Interference at Internal Standard retention time with analytes		**0.00**

5.8. Matrix effect results of Repaglinide

The interference between the retention period of analytes and internal-standard was observed using plasma from six separate allots, one of which included lipemic and one hemolytic plasma. The interference during drug retention was determined by comparing blank plasma response with LLOQ response. The interference throughout the retention period of the Internal Standard contrasts with the retrieved Internal Standard response in the LLOQ sample. If the mean drug response is less than 20% in the LLOQ-sample and less than 5% in the Internal Standard, the reaction of the interfering constituent will be considered acceptable. Tables 5.12 and 5.13 show the outcomes.

Table 5.12. Repaglinide's matrix effect in the absence of matrix ion

Sample allots	LQC			HQC		
	Repaglinide Area	**Internal Standard Area**	**Area ratio**	**Repaglinide Area**	**Internal Standard Area**	**Area ratio**
1.	17345	18067	0.95	43453	43446	0.93
2.	17853	18544	0.98	45484	44136	0.92
3.	17458	18452	1.09	43136	43768	0.94
4.	17802	18550	1.53	45376	44266	0.94
5.	17794	17156	1.86	44521	45643	0.97
6.	17822	18256	1.94	44624	43108	0.91
Mean			**0.955**			**0.944**

Table 5.13. Repaglinide's matrix effect in the absence of plasma lots matrix ion

Plasma allots	LQC				HQC			
	Repaglinide Area	**Internal Standard Area**	**Area ratio**	**N-MF**	**Repaglinide Area**	**Internal Standard Area**	**Area ratio**	**N-MF**
86015P	17203	18554	0.89	0.948	45768	45123	0.964	1.451
86016P	17655	18458	0.94	0.946	45069	44658	0.965	1.654
86017P	17985	17835	0.49	1.256	45489	46825	0.965	1.874
86018P	16967	17526	0.96	1.584	45361	44362	0.993	1.125
86019P	17845	18164	0.95	1.650	44652	45521	0.986	1.032
86020P	17685	18167	0.97	1.350	44362	45542	0.987	1.364
Lipemic	17684	18256	0.94	1.254	45201	46325	0.948	1.542
Haemolytic	17684	18104	0.90	1.564	44320	45247	0.947	1.325
Mean				**1.354**				**1.105**
SD				**1.25**				**0.155**
% CV				**2.59**				**1.564**
N				**6**				**6**

5.9. Recovery study results of Repaglinide

The crest response of extracted and non-extracted samples was compared to show the extraction effectiveness of an analytical procedure. Six samples of HQC, MQC and LQC were freshly prepared, then processed and injected using an internal standard. Six sets of HQC, MQC and LQC with internal standards were spiked into non-extracted samples and instilled into 18 blank matrix samples. Six non-extracted samples were made for each of the three levels by spiking 10 µl of analytes and 10 µl of the internal standard into extracted blank plasma. The mean percent recovery was estimated, and a gap of less than 25% should not exist between the greatest and lowest percent recovery. Tables 5.14 to 5.16 show the findings of the recovery study.

Table 5.14. Repaglinide recovery study

Run	LOQ		MQC		HQC	
	Extracted sample crest area	Non-Extracted sample crest area	Extracted sample crest area	Non-Extracted sample crest area	Extracted sample crest area	Non-Extracted sample crest area
1.	17256	19138	22135	27235	42178	43654
2.	17045	19655	22653	27568	42532	42045
3.	18835	19132	23065	27659	42045	45646
4.	17138	19358	22436	28035	42725	42353
5.	17658	18665	22364	27865	42856	41554
6.	17356	18968	22585	28837	43189	42839
Mean	**17358.06**	**19356.11**	**22356.51**	**27365.25**	**42135.5**	**42436**
SD	**11.63**	**23.35**	**115.10**	**56.08**	**351.545**	**514.74**
%CV	**2.58**	**0.153**	**2.102**	**1.823**	**1.071**	**1.02**
N	**6**	**6**	**6**	**6**	**6**	**6**
%R	**96.09**		**96.32**		**97.23**	

Table 5.15. Internal Standard recovery study

Level of concentration	Extracted samples crest area	Non extracted samples crest area
1LQC	17239	18538
2LQC	17756	18130
3LQC	17564	19435
4LQC	17365	18733
5LQC	17954	18007
6LQC	17230	18235
7MQC	22745	26304
8MQC	22535	26119
9MQC	22242	25634
10MQC	22923	26796
11MQC	23456	26632
12MQC	22953	26135
13HQC	42845	44045
14HQC	42535	44135
15HQC	42865	43146
16HQC	41586	44186
17HQC	42176	44045
18HQC	43546	44746
Mean	**27639.72**	**29611.16**
SD	**10800.43**	**10699.22**
%CV	**37.17**	**39.45**
% Recovery	**95.14**	

Table 5.16: Recovery study of Repaglinide analytes

Analytes	Repaglinide
Mean overall recovery	95.44
% Difference	1.08
N	3

5.10. Ruggedness study results of Repaglinide

In order to verify the robustness of the developed method, one batch of precision and accuracy samples was given and analysed using various columns from the same firm and variable reagent allotments. Table 5.17 shows the findings of the ruggedness study.

Table 5.17. Ruggedness study of Repaglinide

Run	LLOQ		LOQ		MQC		HQC	
	10 ng/ml	Accuracy %	40 ng/ml	Accuracy %	100 ng/ml	Accuracy %	200 ng/ml	Accuracy %
1.	9.46	94.60	37.56	93.90	95.46	95.46	189.56	94.78
2.	9.04	90.46	35.46	90.16	93.26	93.26	197.43	98.71
3.	8.94	89.4	36.45	92.49	92.46	92.46	191.26	95.63
4.	9.37	93.7	35.26	90.07	92.49	92.49	194.53	97.26
5.	9.02	90.2	36.86	92.88	94.56	94.56	194.35	97.03
6.	9.06	90.6	38.56	94.53	91.61	91.61	192.48	96.05
Mean	**9.148**		**36.69**		**93.30**		**193.26**	
SD	**0.193**		**1.147**		**1.45**		**2.53**	
%CV	**2.32**		**3.42**		**1.55**		**1.43**	
% Nominal	**91.49**		**92.33**		**93.30**		**96.57**	

5.11. Dilution integrity results of Repaglinide

By doubling the highest standard concentration by 1.5 times, 12 sets of QC stock solutions were made to evaluate the dilution-integrity of the established procedure. Six dilution-integrity samples were created by diluting twice, while the remaining six samples were created by diluting four times. To determine concentrations, these samples were multiplied by permissible dilution factors of two (for 2 times dilution) and four (for 4 times dilution). At least 67% (four out of six) of the quality control samples should be 15 % of the nominal value for each dilution level. Table 5.18 shows the results.

Table 5.18. Repaglinide dilution integrity

Run	Dilution of 2 times		Dilution 4 times	
	100 ng/ml	Accuracy %	100 ng/ml	Accuracy %
1.	96.75	96.75	98.35	98.35
2.	96.57	96.57	96.79	96.79
3.	92.45	92.45	97.67	97.67
4.	98.56	98.56	97.38	97.38
5.	89.68	89.68	97.64	97.64
6.	96.06	96.06	98.38	98.38
Mean	**95.01**		**97.70**	
SD	**3.29**		**0.55**	
% CV	**3.46**		**6.20**	
% Nominal	**95.01**		**97.70**	

5.12. Stability studies of Repaglinide

Quality control samples were prepared at the low as well as high levels and analysed for the stability study (room temperature, refrigerator, and freeze-thaw). Stability sample concentration was determined using concentration-response linearity data.

a. Stability study of stock solution at room temperature

It was done using a stock solution for at least six hours. The analyte stock solution and internal standard solution were freshly prepared. The stability samples (stock solution) and comparative samples (new stock solution) were diluted to their final concentrations, which remain parallel to the final MQC analytes and internal standards. The percent stability was measured after six duplicates of fresh and comparative samples were instilled. It should be between 95% and 105 %, with a less than 10% CV. Tables 5.19 and 5.20 show the results.

Table 5.19. Repaglinide stock solution stability at room temperature

Run	Crest area of stability stock solution kept 6 hours ambient temperature at MQC level.	Crest area comparison standard solution at the MQC level
1.	23123	22183
2.	22145	22951
3.	23085	22224
4.	23884	22745
5.	22873	22154
6.	23201	22823
Mean	**23051.53**	**22513.53**
SD	**512.38**	**364.16**
% CV	**2.43**	**1.61**
% Stability	**96.46**	

Table 5.20. Internal Standard stability at room temperature stock solution

Run at MQC level	Crest area of stability stock solution kept 6 hours room temperature	Crest area of comparison standard solution
1.	22139	23227
2.	23895	22122
3.	22184	23825
4.	22839	22943
5.	22011	23007
6.	23775	22083
Mean	**22807**	**22867**
SD	**773.49**	**611.33**
% CV	**3.71**	**2.92**
% Stability	**96.74**	

b. Stability study of stock solution at refrigeration

Six duplicates of the stock solution were made and stored at 2 to 8°C for four days to achieve this level of stability. A comparison sample (new standard stock solution) was prepared on the assessment day parallel to the final MQC analytes concentration with the final internal standard concentration in the reconstituted solution. All samples of stability and comparability were immediately injected. For analytes and internal standards, the percent stability was computed, and it had to be between 95-105% with a percent CV of less than 10%. Tables 5.21 and 5.22 provide the results.

Table 5.21. Repaglinide stock solution stability at refrigeration

Run	Crest area of stability stock solution refrigerated at MQC level	Crest area of comparison standard solution at MQC level
1.	22145	23365
2.	23845	22594
3.	22103	23648
4.	22453	22031
5.	22597	23034
6.	23654	22560
Mean	**22799.53**	**22872.46**
SD	**694.95**	**540.58**
%CV	**3.33**	**592.43**
% Stability	**96.08**	

Table 5.22. Internal Standard stock solution stability at refrigeration

Run at MQC level	Refrigerator stability stock solution (Crest area)	Comparison standard solution (Crest area)
1.	22351	22652
2.	22034	22351
3.	23650	23984
4.	22656	22534

5.	23354	23873
6.	22483	22523
Mean	**22745.53**	**22986**
SD	**566.66**	**672.83**
% CV	**2.72**	**3.20**
% Stability	**97.36**	

c. Stability study at freeze-thaw

It took four freeze-thaw rounds. Six LQC and HQC duplicates were frozen at -70^{o}C. Six samples were thawed and refrozen after 24 hours. After 12 hours, residual samples are removed and refrozen. All samples underwent 4 cycles. Stability samples were quantified for LQC and HQC samples. Mean percentage fluctuations for LQC and HQC were 2.35% and 2.54%, respectively. This result met the condition of mean percent variation within 15%, as stated in table 5.23.

Table 5.23. Repaglinide stability study at freeze-thaw

RUN	**LQC**		**HQC**	
	40 ng/ml	**Accuracy %**	**200 ng/ml**	**Accuracy %**
1.	37.56	92.23	194.88	97.44
2.	36.56	91.53	192.86	95.15
3.	35.46	90.86	194.31	97.56
4.	35.56	90.49	193.46	96.08
5.	35.69	90.30	195.68	98.22
6.	35.79	90.48	193.56	96.15
Mean	**35.96**		**193.21**	
SD	**0.547**		**1.235**	
% CV	**2.356**		**2.54**	
% Nominal	**90.53**		**95.76**	
% Stability	**92.35**		**93.02**	

5.13. Repaglinide Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD measures the analytic method's ability to find the lowest analyte strength. The LOQ is the lowest analyte strength to quantify with admissible precision and accuracy. It has been estimated based on the ratio of signal-to-noise. LOQ was determined by calculating the concentration of three spiked calibration standards with less than 20% reproducible and accuracy of 80% to 120%. By increasing plasma/injection quantity, sensitivity may be increased. Table 5.24 shows the findings.

Conclusion

The current approach for detecting Repaglinide in rabbit plasma is sensitive, simple, precise, accurate and specific. The linear calibration curve from 10 to 300 ng/ml shows that the approach is suitable for PK studies.

Table 5.24: LOD & LOQ of Repaglinide

Conc (ng/ml)	Morning			Afternoon			Evening			Avg	SD	RSD	(ng/ml)	
10	0.17	0.172	0.171	0.171	0.173	0.17	0.172	0.172	0.171	0.1713	0.001	0.58366	**LOD**	**LOQ**
20	0.39	0.38	0.37	0.38	0.36	0.35	0.38	0.38	0.38	0.3744	0.01236	3.30098	1.498	4.9931
40	0.79	0.77	0.75	0.77	0.74	0.75	0.77	0.77	0.77	0.7644	0.01509	1.97428		
80	1.56	1.55	1.54	1.53	1.52	1.53	1.55	1.55	1.53	1.54	0.01323	0.85901		
100	1.96	1.95	1.97	1.96	1.95	1.96	1.95	1.95	1.96	1.9567	0.00707	0.36138		
200	3.9	3.89	3.87	3.86	3.87	3.86	3.89	3.89	3.86	3.8767	0.01581	0.40786		
300	5.3	5.2	5.1	5.22	5.21	5.22	5.2	5.2	5.22	5.2078	0.05094	0.97807		

5.14. An overview of validated HPLC parameters for Repaglinide estimation

Table 5.25: Validated HPLC parameters for Repaglinide estimation

Parameters	Criteria	Acceptance criteria	Observations	Passes/Fails
Linearity	Coefficient of correlation (r^2)	≥0.98	0.9992	Passes
Range	Concentration range	-	10 - 300 ng/ml	-
Accuracy	% Nominal	±15%	94.39 – 97.36 %	Passes
Precision	% CV	±15%	1.06 – 2.53 %	Passes
Selectivity	% CV of Repaglinide	-	0.86 %	
	% CV of Internal Standard	-	0.26 %	
Specificity	% of Interferences during the analyte retention time in the presence of Internal Standard	0.00	0.00	Passes
Recovery	Mean overall % recovery	-	95.44	Passes
	Mean overall recovery % difference	The percentage difference between the highest and lowest percentage recovery should not be more than 25%.	1.08%	Passes

Parameters	Criteria	Acceptance criteria	Observations	Passes/Fails
Ruggedness	% Nominal	-	91.49 - 96.57 %	Passes
Dilution Integrity	Two dilution levels of % nominal	Each dilution level at least 67%	95.01 %	Passes
	Four dilution levels of % nominal		97.70 %	Passes
Stability	% Room temperature stability	95 -105 %	96.46 %	Passes
	% Refrigerator stability	95 - 105 %	96.74 %	Passes
	% Freeze-Thaw Stability	95 - 105 %	92.35 - 93.02 %	Passes
LOD	Less than first conc	<10 ng/ml	1.498 ng/ml	Passes
LOQ	Less than first conc	<10 ng/ml	4.9931 ng/ml	Passes

Discussion

1. The regression equation for the bioanalytical study was Y= 0.0177x - 0.053, with r^2 = 0.9992 as the correlation coefficient.

2. The percent mean recovery for Repaglinide in LQC, MQC and HQC was 96.09%, 96.32%, and 97.23%, respectively.

3. The method is accurate, precise, and rugged with % CV ± 15% when tested at MQC, HQC, and LQC levels.

4. In the presence of an Internal Standard, there is no percent of interferences during the analyte retention time. The result shows no drug-excipient interference, and this method adequately resolves the drugs.

5. The stability was assessed at different levels. Room temperature stability, refrigerator stability and freeze-thaw stability studies showed that the compound under analysis is stable under test conditions.

Hence this method was considered suitable and was successfully applied to PK studies.

6. BIO-ANALYTICAL METHOD DEVELOPMENT FOR SITAGLIPTIN ESTIMATION IN RABBIT PLASMA

6.1. Optimized chromatographic conditions

- **Mobile phase:** Acetonitrile: 0.05%TEA (triethanolamine) at pH 6.5
  Ratio: (20:80)
- **HPLC used:**

  RP18 HPLC system Symmetry shield (for water-soluble)
  With 2998 Photo Diode Areditector, quaternary pump with the software of empower 3.
  C-18 HS Column (250nm x 4.6mm x5 µm)
  BDS C-18 guard column (20mm x 4 mm)
- **Flow rate**: 1 ml/min.
- **Internal Standard used:** Rosiglitazone
- **Retention time of Sitagliptin**: 5.232 min
- **Retention time of Rosiglitazone:** 6.903 min

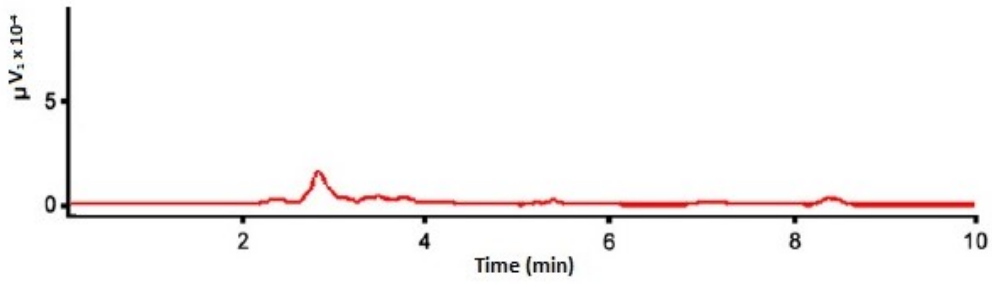


Figure 6.1. ***Blank plasma sample chromatogram***

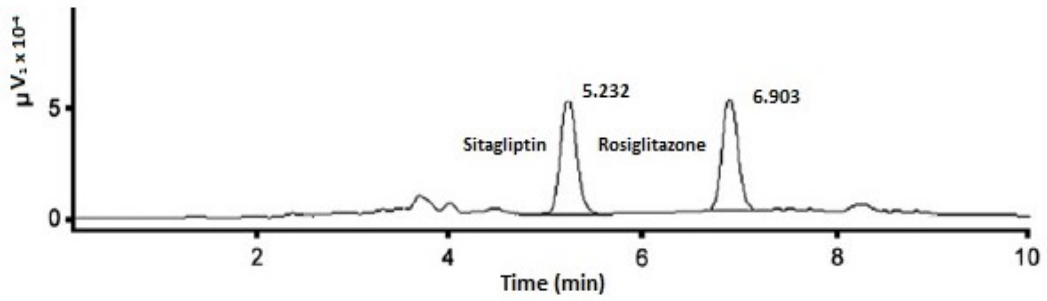


Figure 6.2. ***Sitagliptin in spiked human plasma with Internal Standard of optimized chromatogram***

6.2. Preparation of Sitagliptin stock and working solution

To obtain sitagliptin 1000 µg/ml, 100 mg of sitagliptin was dissolved in 10 ml of acetonitrile, and the solution was diluted to 100 ml with the mobile phase. To get sitagliptin 100 µg/ml, 10 ml of the above solution was withdrawn, and the volume was diluted up to 100 ml. Now, this was a stock solution. This stock solution was used to make working solutions with concentrations of 1, 2, 4, 8, 16, 32, and 64 µg/ml.

6.3. Calibration curve and quality control sample preparation

Aliquants of blank human-plasma (0.95 ml) were spiked with aliquants (0.05 ml) of prepared working solutions (2, 4, 8, 16, 32, and 64 µg/ml) to establish calibration-curve standards in the range of 100, 200, 400, 800, 1600, and 3200 µg/ml. Similarly, from the working solutions, various quality control samples were made. Tables 6.1 and 6.2 provide further information.

Table 6.1. Preparation of calibration curve standard for Sitagliptin

Working solution utilized (µg/ml)	Aliquant volume withdrawn (ml)	Human plasma volume added (ml)	Final volume (ml)
2	0.05	0.95	100
4	0.05	0.95	200
8	0.05	0.95	400
16	0.05	0.95	800
32	0.05	0.95	1600
64	0.05	0.95	3200

Table 6.2. Preparation of quality control standard samples of Sitagliptin

Working solution utilized (µg/ml)	Volume of aliquant withdrawn (ml)	Volume of human plasma added (ml)	Obtained final concentration (ng/ml)	Level
2	0.05	0.95	100	LLOQ
5	0.05	0.95	250	LQC
20	0.05	0.95	1000	MQC
60	0.05	0.95	3000	HQC
80	0.05	0.95	4000	ULOQ

LLOQ-Lower limit of quantification, LQC- Lower quality control, MQC- Middle quality control, HQC- Higher quality control, ULOQ- Upper limit of quantification

6.4. Sample preparation for HPLC analysis

The liquid-liquid extraction method was used to produce the sample. Six 15 ml stoppered test tubes were filled with an aliquant of spiking human plasma (1 ml) and 50 ml of Internal Standard working solution (10 mg/ml) and swirled for 1 minute. 5 ml dichloromethane:diethyl ether (4:6, v/v) was added to this solution. The constituents of tubes were blended in a slope position at 100 strokes per minute on a reciprocating shaker for 30 minutes and centrifuged for 10 minutes at 3000 rpm to isolate the phases. Under a nitrogen stream, the supernatant layer (4 ml) was moved to

another tube and permitted to evaporate for dryness. The residue was then blended with 250 mL of mobile-phase and chromatographically analysed.

The same procedure will be followed for the sample preparation with rabbit plasma for the analysis into the HPLC system to estimate the plasma drug concentrations of Sitagliptin.

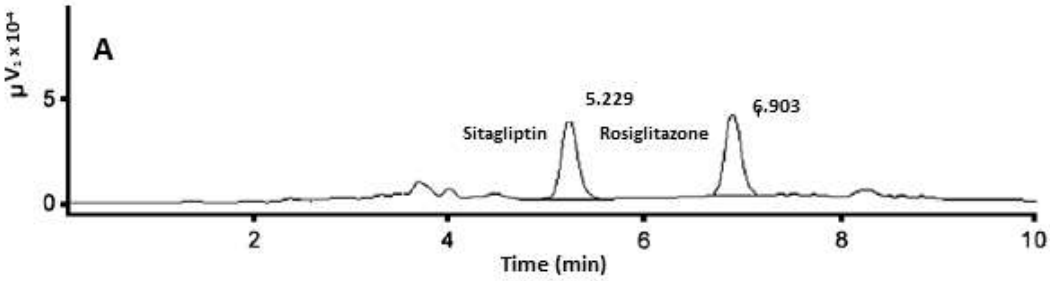


Figure 6.3. ***Chromatogram of Sitagliptin with Internal Standard at LQC level***

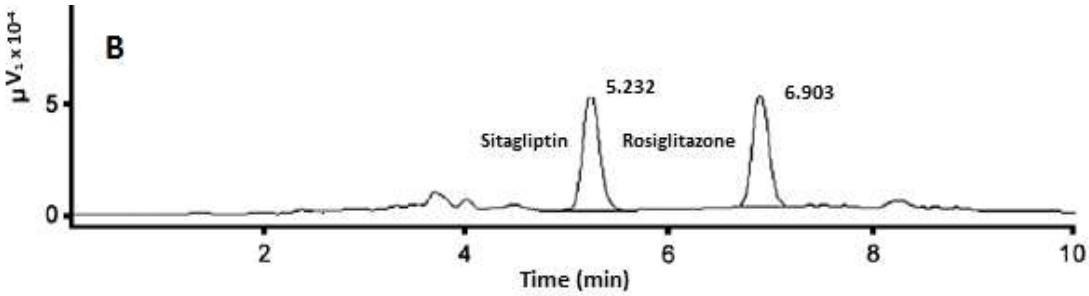


Figure 6.4. ***Chromatograms of Sitagliptin with Internal Standard at MQC level***

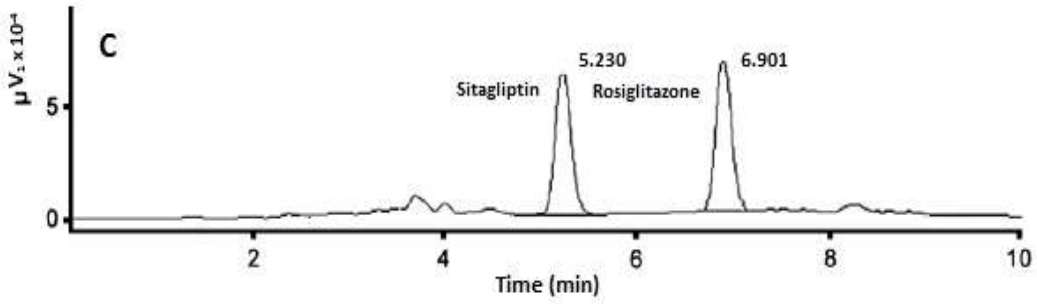


Figure 6.5. ***Chromatogram of Sitagliptin with Internal Standard at HQC level***

6.5. Linearity results of Sitagliptin

The developed method's linearity was validated in a concentration between 100 – 3200ng/ml. Human plasma was used to produce and process the six calibration curve samples. The calibration curve was drwan using a regression calculation with a weighting factor of 1/(concentration ratio)^2 of the medication to internal standard strength to get the proper tally for the concentration/response relation. Linearity acceptability requirement, the r^2 (coefficient of correlation) should be less than 0.98. Table 6.3 and figure 6.6 show the results.

Table 6.3. Area ratios from calibration experiments of Sitagliptin

Level of calibration standards	Amount of drug (ng/ml)	Area ratio (mean ± SD, n=6)
1CC	100	0.13 ± 0.02
2CC	200	0.25 ± 0.02
3CC	400	0.48 ± 0.01
4CC	800	0.86 ± 0.02
5CC	1600	1.79 ± 0.08
6CC	3200	3.83 ± 0.34

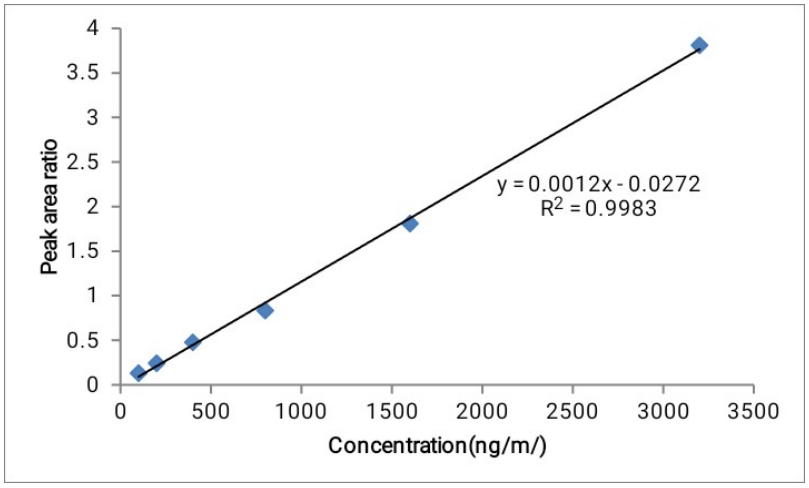


Figure 6.6. Linearity graph of Sitagliptin

6.6. Results of accuracy and precision of Sitagliptin

Several QC samples at LOQ, MQC, and HQC were used in six replicates to estimate intra- and inter-day precision and accuracy. For each replicate, the concentrations in these levels were computed, followed by standard deviation, % CV for precision, and % nominal for accuracy.

The acceptance criteria for accuracy (% nominal) are ±15% and ±20% only for the LLOQ level, and precision (% CV) is ±15% and ±20% only for the LLOQ sample. Tables 6.4 to 6.8 show the intraday and inter day outcomes.

Table 6.4. Day 1 Interday accuracy and precision of Sitagliptin

Day 1	LOQ		MQC		HQC	
	250 ng/ml	Accuracy %	1000 ng/ml	Accuracy %	3000 ng/ml	Accuracy %
1	243.02	97.2	956.52	95.65	2954.45	97.57
2	247.12	98.88	895.23	89.5	2856.56	95.23
3	242.58	97.03	945.22	94.96	2754.02	94.08
4	245.66	96.23	932.38	94.05	2789.5	94.86
5	244.09	98.03	965.52	97.85	2865.23	96.59
6	251.89	102.12	900.77	90.56	2784.25	94.55
Mean	**245.72**		**932.6**		**2834**	
SD	**3.158**		**26.53**		**6688**	
%CV	**1.29**		**2.85**		**2.36**	
% Nominal	**98.56**		**94.59**		**95.94**	
N	**6**		**6**		**6**	

Table 6.5. Day 2 Interday accuracy and precision of Sitagliptin

Day2	LOQ		MQC		HQC	
	250 ng/ml	Accuracy %	1000 ng/ml	Accuracy %	3000 ng/ml	Accuracy %
1	245.68	96.23	945.22	94.96	2789.78	94.86
2	244.09	98.78	932.38	94.05	2865.65	96.59
3	251.83	102.12	965.52	97.85	2784.78	94.55
4	247.12	97.27	956.52	90.56	2954.77	97.57
5	242.57	98.83	895.23	95.65	2856.02	95.23
6	245.66	97.08	900.89	89.5	2754.56	94.08
Mean	**243.89**		**938.82**		**2796.56**	
SD	**2.95**		**12.58**		**4541**	
%CV	**1.8**		**3.54**		**2.98**	
% Nominal	**97.65**		**93.65**		**95.35**	
N	**6**		**6**		**6**	

Table 6.6. Day 3 Interday accuracy and precision of Sitagliptin

Day 3	LOQ		MQC		HQC	
	250 ng/ml	Accuracy %	1000 ng/ml	Accuracy %	3000 ng/ml	Accuracy %
1	245.68	96.54	956.53	95.65	2954.45	94.85
2	254.57	97.78	896.23	89.5	2856.56	95.54
3	251.03	103.12	945.29	94.93	2754.02	94.78
4	245.17	97.27	931.38	94.56	2789.5	97.57
5	246.53	98.86	965.65	97.85	2865.23	95.23
6	247.96	97.65	903.78	90.78	2784.25	94.34
Mean	**241.8**		**956.6**		**2845**	
SD	**2.65**		**26.03**		**6645**	
%CV	**1.76**		**2.35**		**2.36**	
% Nominal	**97.75**		**94.49**		**96.94**	
N	**6**		**6**		**6**	

Table 6.7. Day 4 Interday accuracy and precision of Sitagliptin

Day 4	LOQ		MQC		HQC	
	250 ng/ml	Accuracy %	1000 ng/ml	Accuracy %	3000 ng/ml	Accuracy %
1	243.65	96.45	945.22	94.96	2767.78	95.54
2	243.01	98.89	932.38	94.05	2898.65	96.55
3	251.83	102.76	965.52	97.85	2784.78	96.5
4	247.34	96.56	958.52	90.56	2954.77	97.65
5	242.5	98.8	897.23	95.65	2856.02	95.35
6	244.66	98.45	934.89	89.5	2776.56	96.45
Mean	**241.89**		**978.82**		**2745.56**	
SD	**2.85**		**12.79**		**4591**	
%CV	**1.76**		**3.66**		**2.24**	
% Nominal	**97.65**		**93.65**		**96.39**	
N	**6**		**6**		**6**	

Table 6.8. Intraday accuracy and precision of Sitagliptin

Sl. No	LOQ		MQC		HQC	
	250 ng/ml	Accuracy %	1000 ng/ml	Accuracy %	3000 ng/ml	Accuracy %
1	244.25	94.56	975.51	97.23	2889.28	95.82
2	241.24	89.32	968.54	96.61	2745.65	96.64
3	248.65	99.54	897.98	92.65	2757.78	94.36
4	247.62	98.60	933.22	94.60	2964.77	97.67
5	242.90	93.09	964.54	95.67	2880.52	95.09
6	245.54	94.32	887.28	89.61	2776.56	94.46
Mean	**244.83**		**934.76**		**2798.33**	
SD	**3.56**		**4.51**		**3.65**	
%CV	**1.09**		**2.61**		**4.55**	
% Nominal	**97.52**		**93.56**		**95.64**	
N	**6**		**6**		**6**	

6.7. Results of selectivity study of Sitagliptin

Analyte selectivity was assessed to identify Internal Standard interference during analyte retention period. Six blank matrix internal-standard copies were injected, and if an analyte area was identified, that was rivalled with the analyte's mean area obtained with injected LLOQ strength. Internal standard selectivity was also tested for six blank matrix duplicates with drugs instilled at the ULOQ level. The resulting internal-area standard's was then compared with the mean internal-area standard obtained at the LLOQ level. Tables 6.9 to 6.11 show the results.

Table 6.9. Crest area of Sitagliptin and area of Internal Standard

Sample Id	Sitagliptin (analyte) crest area	Internal standard crest area
LLOQ1 Selectivity	12576	13038
LLOQ2 Selectivity	11208	13382
LLOQ3 Selectivity	12062	14252
LLOQ4 Selectivity	13791	14572
LLOQ5 Selectivity	12058	13890
LLOQ6 Selectivity	11192	13054
Mean	**12149.4**	**13444.3**
SD	**773.27**	**448.33**
%CV	**0.56**	**0.13**

Table 6.10. Analyte Selectivity Study of Sitagliptin

Sample Id	Area of Sitagliptin	Area of IS
(Internal Standard + Blank)-1	0	15243
(Internal Standard + Blank)-2	0	13152
(Internal Standard + Blank)-3	0	13956
(Internal Standard + Blank)-4	0	14393
(Internal Standard + Blank)-5	0	15075
(Internal Standard + Blank)-6	0	14837
Sitagliptin (analyte) with an Internal Standard mean response	**0.00**	
Sitagliptin (analyte) in LLOQ selectivity mean response	**12149.5**	
% of interference at analyte retention time with Internal Standard	**0.00**	

Table 6.11. Internal standard selectivity study of Sitagliptin

Sample Id	Area of Sitagliptin	Area of Is
ULOQ1 Sitagliptin	461092	0
ULOQ2 Sitagliptin	458653	0
ULOQ3 Sitagliptin	452591	0
ULOQ4 Sitagliptin	448832	0
ULOQ5 Sitagliptin	456512	0
ULOQ6 Sitagliptin	459821	0
Internal standard with sitagliptin (analytes) means response.		**0.00**
Internal standard in LLOQ selectivity means response.		**13764.6**
% of interference at Internal Standard retention time with analytes		**0.00**

6.8. Matrix effect results of Sitagliptin

The interference between analyte retention periods and Internal Standards was found using plasma from six different allotments, one of which included lipemic and one hemolytic plasma. The interference during drug retention was determined by comparing blank plasma response with LLOQ response. The interference throughout the retention period of the Internal Standard was compared to the retrieved internal-standard response in the LLOQ-sample. If the mean drug response in the LLOQ-sample is less than 20% and less than 5% in the Internal Standard, the reaction of the interfering constituent is considered acceptable. Tables 6.12 and 6.13 show the outcomes.

Table 6.12. Sitagliptin's matrix effect in the absence of matrix ion

Sample allots	LQC			HQC		
	Sitagliptin area	Internal Standard area	Area ratio	Sitagliptin area	Internal Standard area	Area ratio
1.	17333	18789	0.94	42453	43455	0.95
2.	18870	19543	0.93	45224	44117	0.92
3.	18856	18452	1.07	45136	43764	0.97
4.	16844	18566	0.92	46330	44245	0.90
5.	17279	18156	0.93	44585	45648	0.95
6.	17869	19248	0.91	47624	43156	0.94
Mean			**0.955**			**0.946**

Table 6.13. Matrix effect of Sitagliptin in the presence of matrix ion

Plasma allots	LQC				HQC			
	Sitagliptin area	Internal Standard area	Area ratio	N-MF	Sitagliptin area	Internal Standard rea	Area ratio	N-MF
23015P	16223	18569	0.89	0.956	43729	45123	0.964	1.011
23016P	17845	18334	0.95	1.014	42039	44112	0.958	0.986
23017P	16845	18859	0.86	0.924	42839	43129	0.989	1.034
23018P	16954	17526	0.94	1.024	43129	42092	0.987	1.037
23019P	17659	18177	0.94	1.010	42092	43209	0.988	1.032
23020P	17122	18115	0.93	0.974	42339	41835	0.976	1.028
Lipemic	17345	18223	0.97	0.994	43226	42623	0.976	1.029
Haemolytic	17765	18884	0.99	0.985	42101	44227	0.969	0.989
Mean				**0.979**				**1.23**
SD				**0.045**				**0.057**
% CV				**3.99**				**1.837**
N				**6**				**6**

6.9. Recovery results of Sitagliptin

In this study, the crest response of extracted and non-extracted samples was compared to demonstrate the extraction efficacy of an analytical procedure. Six samples of LQC, MQC, and HQC were newly produced, processed using an Internal Standard, and injected. Non-extracted samples were spiked with six sets of each LQC, MQC, and HQC with internal standard and injected into 18 blank matrix samples. Six non-extracted samples of each level were prepared by spiking 10 µl of analytes and 10 µl of Internal Standard in extracted blank-plasma. The mean percent recovery was estimated, and a gap of less than 25% should not exist between the greatest and lowest percent recovery. Tables 6.14 to 6.16 show the findings of the recovery research.

Table 6.14. Recovery study of Sitagliptin

RUN	LOQ		MQC		HQC	
	Extracted sample crest area	Non-Extracted sample crest area	Extracted sample crest area	Non-Extracted sample crest area	Extracted sample crest area	Non-Extracted sample crest area
1	18286	20138	32155	32889	40178	43093
2	19056	20035	31856	33107	39532	42093
3	18856	19132	32737	33039	40045	45635
4	17129	19388	35678	31137	40725	42353
5	18048	18523	30675	32253	40856	41535
6	19156	17995	32585	33837	39189	42839
Mean	**18428**	**19201**	**31804.83**	**32710.33**	**40167.5**	**42408**
SD	**725**	**839.92**	**1142.10**	**922.08**	**668.545**	**504.75**
%CV	**3.864**	**0.044**	**3.456**	**2.818**	**1.676**	**1.29**
N	**6**	**6**	**6**	**6**	**6**	**6**
%R	**95.46**		**97.45**		**95.44**	

Table 6.15. Recovery study of Internal Standard (Rosiglitazone)

Level of concentration	Extracted samples crest area	Non extracted samples crest area
1LQC	17239	19538
2LQC	18725	19130
3LQC	18039	21435
4LQC	16839	18733
5LQC	17999	19007
6LQC	16133	17235
7MQC	29799	33304
8MQC	28539	32119
9MQC	29229	32634
10MQC	28982	28796
11MQC	30431	31632
12MQC	32935	34135
13HQC	39844	42066
14HQC	38537	39131
15HQC	40884	42121
16HQC	40539	42139
17HQC	38133	39001
18HQC	39555	42761
Mean	**29561.06**	**30.842**
SD	**9277.155**	**9454.61**
%CV	**32.331**	**30.57**
% Recovery	**95.14**	

Table 6.16. Recovery study of Sitagliptin analytes

Analytes	Sitagliptin
Mean overall recovery	96.42
% Difference	1.87
N	3

6.10. Ruggedness results of Sitagliptin

In order to verify the robustness of the developed method, one batch of precision and accuracy samples was given and analysed using various columns from the same firm and variable reagent allotments. Table 6.17 shows the findings of the ruggedness study.

Table 6.17. Ruggedness study of Sitagliptin

RUN	LLOQ		LOQ		MQC		HQC	
	100 ng/ml	Accuracy %	250 ng/ml	Accuracy %	1000 ng/ml	Accuracy %	3000 ng/ml	Accuracy %
1	98.22	98.22	243.02	97.20	945.22	94.96	2789.78	94.86
2	89.65	89.65	247.12	98.88	932.38	94.05	2865.65	96.59
3	92.56	92.56	242.58	97.03	965.52	97.85	2784.78	94.55
4	87.56	87.56	245.66	96.23	956.52	90.56	2954.77	97.57
5	90.56	90.56	244.09	98.03	895.23	95.65	2856.02	95.23
6	91.78	91.78	251.89	102.12	900.89	89.50	2754.56	94.08
Mean	**93.08**		**246.34**		**938.82**		**2756**	
SD	**0.0211**		**0.0556**		**0.1063**		**0.3808**	
%CV	**11.89**		**11.27**		**4.348**		**7.101**	
% Nominal	**94.56**		**102.22**		**95.89**		**95.59**	

6.11. Dilution integrity results of Sitagliptin

By doubling the highest standard concentration by 1.5 times, 12 sets of QC stock solutions were made to evaluate the dilution-integrity of the established procedure. Six dilution-integrity samples were created by diluting twice, while the remaining six samples were created by diluting four times. To determine concentrations, these samples were multiplied by permissible dilution factors of two (for 2 times dilution) and four (for 4 times dilution). At least 67% (four out of six) of the quality control samples should be 15 % of the nominal value for each dilution level. Table 6.18 shows the results.

Table 6.18. Sitagliptin dilution integrity

Run	Dilution of 2 times		Dilution 4 times	
	100 ng/ml	Accuracy %	100 ng/ml	Accuracy %
1	101.23	101.23	96.12	96.12
2	98.56	98.56	95.68	95.68
3	89.56	89.56	96.44	96.44
4	89.44	89.44	96.92	96.92
5	87.65	87.65	94.88	94.88
6	101.24	101.24	96.20	96.20
Mean	**94.22**		**95.95**	
SD	**0.7793**		**0.202**	
% CV	**3.179**		**0.84**	
% Nominal	**98.04**		**96.08**	

6.12. Stability studies of Sitagliptin

For the stability study, quality control samples were prepared at low as well as high levels and analysed (room temperature, refrigerator, and freeze-thaw). Concentration of stability samples was determined using concentration-response linearity data.

a. Stability study of stock solution at room temperature

It was done using a stock solution for at least six hours. The analyte stock solution and Internal Standard solution were freshly prepared. The stability samples (stock solution) and comparative samples (new stock solution) were diluted to their final concentrations, which remain parallel to the final MQC analytes and Internal Standards. The percent stability was measured after six duplicates of fresh and comparative samples were instilled. It should be between 95% and 105 %, with a less than 10% CV. Tables 6.19 and 6.20 show the results.

Table 6.19. Sitagliptin stock solution stability at room temperature

Run	Crest area of stability stock solution kept 6 hours ambient temperature at MQC level	Crest area comparison standard solution at the MQC level
1.	33123	34183
2.	32145	33951
3.	33085	32224
4.	33884	33745
5.	32873	34154
6.	33201	33823
Mean	**33457**	**33650**
SD	**612.07**	**732.44**
% CV	**1.648**	**2.187**
% Stability	**98.06**	

Table 6.20. Internal Standard stability at room temperature stock solution

Run at MQC level	Crest area of stability stock solution kept 6 hours room temperature	Crest area of comparison standard solution
1.	35139	35227
2.	33895	34122
3.	34184	34825
4.	34839	34943
5.	35011	33007
6.	34775	34083
Mean	**34640.50**	**34367.83**
SD	**491.4117**	**808.877**
% CV	**1.4186**	**2.3535**
% Stability	**100.79**	

b. Stability study of stock solution at refrigeration

Six duplicates of the stock solution were made and stored at 2 to 8°C for four days to achieve this level of stability. A comparison sample (new standard stock solution) was prepared on the assessment day parallel to the final MQC analytes concentration with the final internal standard concentration in the reconstituted solution. All samples for comparability and stability were injected immediately. For analytes and Internal Standards, the percent stability was computed, and it had to be between 95-105% with a percent CV of less than 10%. Tables 6.21 and 6.22 provide the results.

Table 6.21. Sitagliptin stock solution stability at refrigeration

Run	Crest area of stability stock solution refrigerated at MQC level	Crest area of comparison standard solution at MQC level
1.	32139	34927
2.	32833	33129
3.	33773	34275
4.	33407	34437
5.	34847	34263
6.	33326	32862
Mean	**33316.5**	**33956.8**
SD	**940.28**	**789.44**
% CV	**2.532**	**2.041**
% Stability	**98.19**	

Table 6.22. Internal Standard stock solution stability at refrigeration

Run at MQC level	Refrigeration stability stock solution (Crest area)	Comparison standard solution (Crest area)
1.	32256	33565
2.	33256	34565
3.	32056	34193
4.	33956	33246
5.	32562	34746
6.	32239	33054
Mean	**32622.7**	**34573**
SD	**732.59**	**669.44**
% CV	**2.202**	**1.593**
% Stability	**96.59**	

3. Stability study at freeze-thaw

It required four cycles of freezing and thawing. Six LQC and HQC duplicates were frozen at -70ºC. Six samples were thawed and refrozen after 24 hours. After 12 hours, residual samples are removed and refrozen. All samples underwent 4 cycles. Stability samples were quantified for LQC and HQC samples. Mean percentage fluctuations for LQC and HQC were 3.84% and 9.69%, respectively. The result met the mean percent variation by 15%, as in table 6.23.

Table 6.23. Sitagliptin stability study at freeze-thaw

RUN	LQC		HQC	
	250 ng/ml	**Accuracy %**	**3000 ng/ml**	**Accuracy %**
1.	251.56	101.56	2856.22	90.56
2.	248.16	98.65	2976.49	98.56
3.	239.59	96.70	2902.06	97.34
4.	241.96	89.51	2930.31	97.00
5.	246.03	94.22	2894.77	96.38
6.	245.26	96.54	2961.11	98.53
Mean	**243.88**		**2936.45**	
SD	**0.0154**		**0.4670**	
% CV	**3.8493**		**9.69**	
% Nominal	**97.44**		**95.76**	
% Stability	**98.26**		**97.45**	

6.13. Sitagliptin Limit of Detection and Limit of Quantification

LOD measures the analytic method's ability to find the lowest analyte strength. The LOQ is the lowest analyte strength to quantify with admissible precision and accuracy. It has been estimated based on the ratio of signal-to-noise. LOQ was determined by calculating the concentration of three spiked calibration standards with less than 20% reproducible and accuracy of 80% to 120%. By increasing plasma/injection quantity, sensitivity may be increased. Table 6.24 shows the findings.

Conclusion

The current approach for detecting Sitagliptin in rabbit plasma is sensitive, simple, precise, accurate and specific. The linear calibration curve from 100 to 3200 ng/ml shows that the approach is suitable for PK studies.

Table 6.24. LOD & LOQ of Sitagliptin

Conc (ng/ml)	Morning			Afternoon			Evening			Avg	SD	RSD	(ng/ml)	
100	0.13	0.132	0.13	0.131	0.132	0.132	0.13	0.132	0.13	0.131	0.001	0.76336	**LOD**	**LOQ**
200	0.25	0.24	0.24	0.24	0.25	0.24	0.23	0.24	0.25	0.2422	0.00667	2.75229	8.592	28.641
400	0.48	0.47	0.49	0.48	0.47	0.46	0.48	0.47	0.49	0.4767	0.01	2.0979		
800	0.86	0.85	0.84	0.82	0.81	0.82	0.86	0.81	0.84	0.8344	0.02007	2.40511		
1600	1.79	1.8	1.81	1.82	1.83	1.81	1.79	1.83	1.81	1.81	0.015	0.82873		
3200	3.83	3.82	3.81	3.8	3.81	3.83	3.83	3.81	3.81	3.8167	0.01118	0.29293		

6.14. An Overview of validated HPLC parameters for Sitagliptin estimation

Table 6.25. Validated HPLC parameters for Sitagliptin estimation

Parameters	Criteria	Acceptance criteria	Observations	Passes/Fails
Linearity	Coefficient of correlation (r^2)	≥0.98	0.9983	Passes
Range	Concentration range	-	100-3200 ng/ml	-
Accuracy	% Nominal	±15%	93.56 - 98.56 %	Passes
Precision	% CV	±15%	1.09 - 4.55 %	Passes
Selectivity	% CV of Sitagliptin	-	0.56 %	-
	% CV of Internal Standard	-	0.13 %	-
Specificity	% of Interferences at the retention time of the analyte in the presence of Internal Standard	0.00	0.00	Passes
Recovery	Mean overall % recovery	-	96.42	Passes
	Mean overall recovery % difference	% of difference should not be more than 25% between the highest and lowest % recovery	1.87 %	Passes
Ruggedness	% Nominal	-	94.56 - 102.22 %	Passes
Dilution Integrity	Two dilution levels of % nominal	Each dilution level at least 67%	98.04 %	Passes
	Four dilution level of % nominal		96.08 %	Passes
Stability	% Room temperature stability	95 -105 %	98.06 %	Passes

	% Refrigerator stability	95 - 105 %	98.19 %	Passes
	% Freeze-Thaw stability	95 - 105 %	97.45 - 98.26 %	Passes
LOD	Less than first conc	<100 ng/ml	8.592 ng/ml	Passes
LOQ	Less than first conc	<100 ng/ml	28.641 ng/ml	Passes

Discussion

1. The regression equation for the analysis was Y= 0.002x - 0.0272 with coefficient of correlation, $r^2 = 0.9983$.

2. The percent mean recovery for Sitagliptin in LQC, MQC, and HQC was 95.46%, 97.45%, and 95.44%, respectively.

3. The method is accurate, precise, and rugged with % CV ± 15% when tested at MQC, HQC, and LQC levels.

4. There is no % of interferences at the retention time of the analyte in the presence of internal standard. The study result indicates that this method has no drug-excipient interference, and the drugs are properly resolved.

5. The stability was assessed at different levels. Room temperature stability, refrigerator stability, and freeze-thaw stability studies showed that the compound under analysis is stable under test conditions.

Hence this method was suitable for further PK studies.

7. INTERACTION STUDY OF REPAGLINIDE WITH PIPERINE

7.1. PD interaction study of Repaglinide with Piperine in Rats

7.1.1 PD interaction in Normal Rats (Effect on Blood glucose levels)

Table 7.1. Blood glucose levels in Normal Rats.

Type of Treatment	Blood Glucose Levels (mg/dl)				
	Day	Vehicle	Piperine	Repaglinide	Piperine + Repaglinide
	0	82.00 ±5.48	85.83 ± 4.22	86.33± 6.12	85.50± 4.09
SDT	**1**	81.33 ± 6.06	83.00 ± 2.97	80.50± 4.76	68.50± 5.65 ^{c,**}
MDT	**3**	83.00 ± 9.84	84.67± 3.01	69.42± 7.36 ^{b,**}	55.94± 4.59 ^{c,*}
	7	81.67 ± 6.12	81.00± 3.22	60.59 ± 4.43^{b,***}	52.95± 6.07
	14	82.00 ± 2.19	82.17± 6.01	56.77± 4.25 ^{b,***}	49.13 ± 3.49 ^{c,*}
	21	79.17 ± 3.60	82.00± 4.34	51.26 ± 3.76 ^{b,***}	44.76± 3.51 ^{c,*}

SDT- Single-dose treatment, MDT-Multiple-dose treatment
All values are expressed as mean ± SD (n=6). *p < 0.05; **p < 0.01 and ***p < 0.001.[a] when compared with vehicle control group, [b]when compared with Piperine alone group, [c] when compared with Repaglinide alone group.

Table 7.2. Percent blood glucose reductions in Normal Rats.

Type of Treatment	Percent Blood Glucose Reduction (Mean ± SD)				
	Day	Vehicle	Piperine	Repaglinide	Piperine + Repaglinide
SDT	**1**	-0.76 ±4.91	3.19 ±3.77	6.42 ± 7.68	19.66 ± 8.57 ^{c,*}
MDT	**3**	1.04 ± 7.67	1.23 ± 4.20	19.08 ± 11.90 ^{b,**}	34.32 ± 7. 68 ^{c,*}
	7	-0.41 ±3.34	5.51 ± 4.31	29.53 ± 6.97 ^{b,***}	37.89 ± 8.25
	14	0.28 ± 5.52	4.29 ± 4.81	34.20 ± 3.04 ^{b,***}	42.51 ± 3.6 ^{c,*}
	21	-3.25 ± 4.91	4.37 ± 4.91	40.51 ± 3.97 ^{b,***}	47.5 ± 5.35

SDT- Single-dose treatment, MDT-Multiple-dose treatment
All values are expressed as mean ± SD (n=6). *p < 0.05; **p < 0.01 and ***p < 0.001.[a] when compared with vehicle control group, [b]when compared with Piperine alone group, [c] when compared with Repaglinide alone group.

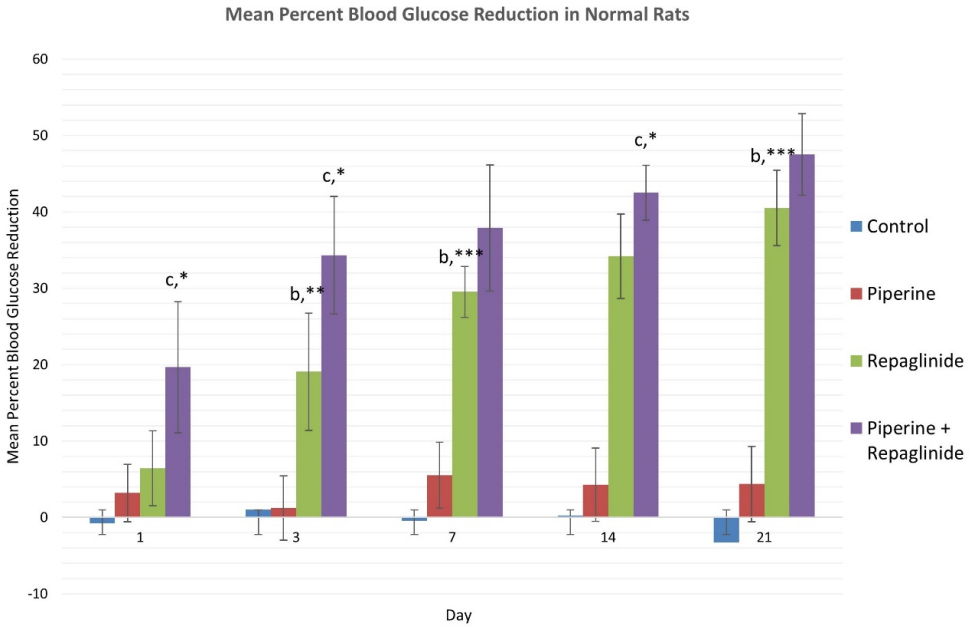


Figure 7.1. Mean percent blood glucose reduction in Normal Rats.

All values are expressed as mean ± SD (n=6). *p < 0.05; **p < 0.01 and ***p < 0.001.[a] when compared with vehicle control group, [b]when compared with Piperine alone group, [c] when compared with Repaglinide alone group.

7.1.2. PD interactions in Diabetic Rats (Effect on Blood glucose levels)

Table 7.3. Blood glucose levels in Diabetic Rats.

Type of Treatment	Blood Glucose Levels (mg/dl)				
	Day	Vehicle	Piperine	Repaglinide	Piperine + Repaglinide
	0	311.26± 6.76	312.73 ± 7.95	309.11 ± 10.80	311.85± 9.22
SDT	**1**	317.50± 9.33	220.23 ± 12.97	207.97± 15.51	184.67 ±10.33[c,*]
MDT	**3**	325.88± 10.18	208.43 ± 7.56	192.94 ± 10.29 [b,*]	176.71 ± 8.64 [c,*]
	7	333.43± 9.08	195.83 ± 10.21	184.00 ± 10.40	163.07 ± 11.55 [c,*]
	14	340.03 12.56	187.50 ± 12.14	166.30 ± 12.08 [b,*]	145.92± 11.05 [c,*]
	21	347.46± 11.12	173.83 ±13.03	157.38 ± 8.97 [b,*]	136.88 ± 5.13 [c,**]

SDT- Single-dose treatment, MDT-Multiple-dose treatment

All values are expressed as mean ± SD (n=6). *p < 0.05; **p < 0.01 and ***p < 0.001.[a] when compared with vehicle control group, [b]when compared with Piperine alone group, [c] when compared with Repaglinide alone group.

Table 7.4. Percent blood glucose reductions in Diabetic Rats.

Type of Treatment	Percent Blood Glucose Reduction (Mean ± SD)				
	Day	Vehicle	Piperine	Repaglinide	Piperine + Repaglinide
SDT	1	-2.00 ± 1.89	29.59 ± 3.62	32.76 ± 3.61	40.74 ± 3.68 ^{c,**}
MDT	3	-4.71 ± 2.77	33.31 ± 2.96	37.56 ± 3.12	42.54 ± 4.72
	7	-7.15 ± 2.91	37.34 ± 3.72	40.46 ± 3.06^{b,***}	47.69 ± 3.8 ^{c,**}
	14	-9.23 ± 2.66	40.03 ± 3.89	46.19 ± 3.6 ^{b,*}	53.12 ± 4.53 ^{c,*}
	21	-11.64 ± 2.178	44.38 ± 4.46	49.01 ± 3.94	56.09 ± 1.66 ^{c,**}

SDT- Single-dose treatment, MDT-Multiple-dose treatment
All values are expressed as mean ± SD (n=6). *p < 0.05; **p < 0.01 and ***p < 0.001.[a] when compared with vehicle control group, [b]when compared with Piperine alone group, [c] when compared with Repaglinide alone group.

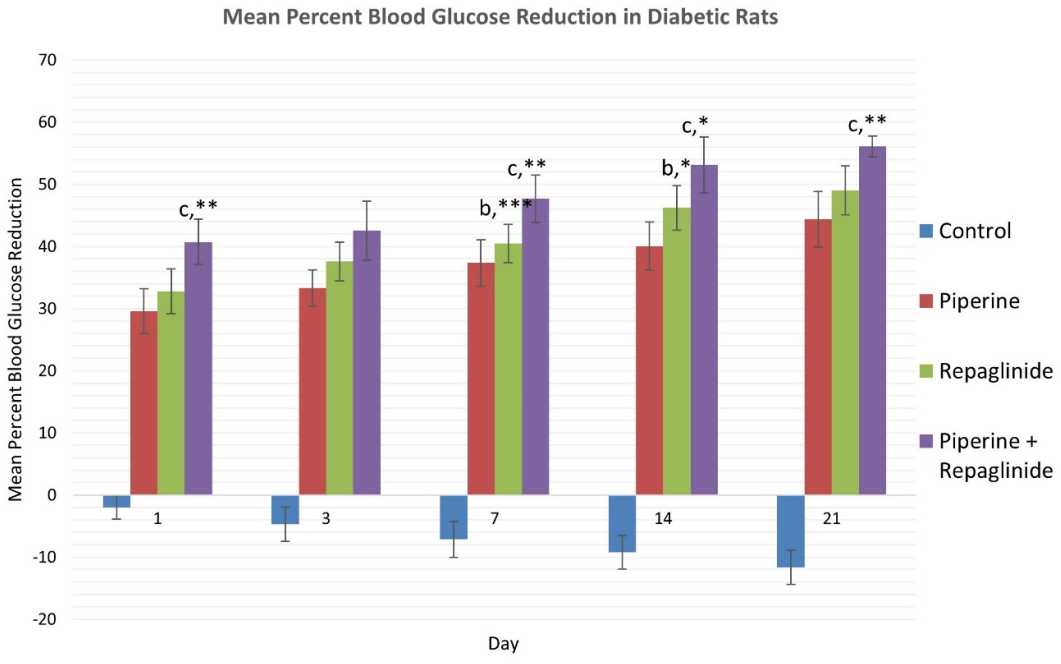


Figure 7.2. Mean percent blood glucose reductions in Diabetic Rats.

All values are expressed as mean ± SD (n=6). *p < 0.05; **p < 0.01 and ***p < 0.001.[a] when compared with vehicle control group, [b]when compared with Piperine alone group, [c] when compared with Repaglinide alone group.

7.1.3. Repaglinide and Piperine Interactions in Normal Rats: A Study of Insulin Levels

Table 7.5. Insulin levels in Normal Rats.

Type of Treatment	Insulin Levels (mIU/L)				
	Day	**Vehicle**	**Piperine**	**Repaglinide**	**Piperine + Repaglinide**
	0	12.29 ± 0.25	11.97 ± 0.30	11.80 ± 0.54	11.72 ± 0.54 ns
SDT	**1**	12.46 ± 0.25	12.09 ± 0.27	16.56 ± 0.29	17.05 ± 0.38 *
MDT	**3**	12.46 ± 0.18	12.69 ± 0.37	16.91 ± 0.43	17.59 ± 0.37 *
	7	12.65 ± 0.14	12.98 ± 0.32	17.49 ± 0.27	17.96 ± 0.24 *
	14	12.76 ± 0.27	13.22 ± 0.38	18.24 ± 0.17	18.80 ± 0.30 *
	21	12.29 ± 0.28	13.79 ± 0.42	18.63 ± 0.21	19.19 ± 0.37 *

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at *p< 0.05 when compared with repaglinide alone

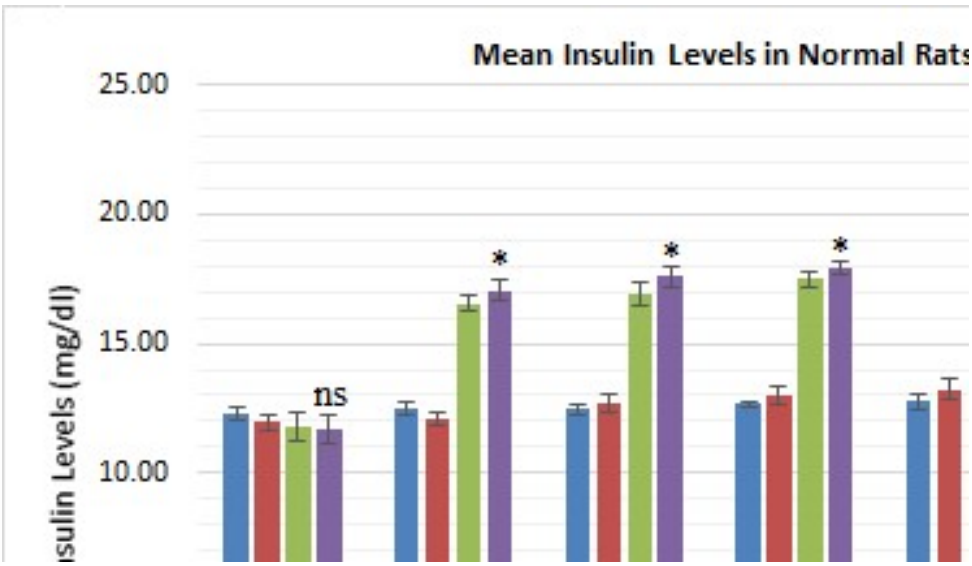


Figure 7.3. Insulin levels in Normal Rats.

ns- non significant, significant at *p< 0.05 when compared with repaglinide alone

7.1.4. Repaglinide and Piperine Interactions in Diabetic Rats: A Study of Insulin Levels

Table 7.6. Insulin levels in Diabetic Rats.

Type of Treatment	Insulin Levels (mIU/L)				
	Day	**Vehicle**	**Piperine**	**Repaglinide**	**Piperine + Repaglinide**
	0	11.92 ± 0.76	11.71 ± 0.42	11.87 ± 0.52	11.61 ± 0.32 ns
SDT	**1**	11.54 ± 0.79	13.50 ± 0.19	13.58 ± 0.21	14.40 ± 0.15 *
MDT	**3**	10.79 ± 0.49	14.86 ± 0.37	14.95 ± 0.62	15.75 ± 0.09 *
	7	10.02 ± 0.38	15.57 ± 0.23	16.58 ± 0.21	17.01 ± 0.17 *
	14	8.69 ± 0.50	16.38 ± 0.26	17.35 ± 0.19	17.98 ± 0.20 *
	21	7.52 ± 0.71	17.39 ± 0.20	18.46 ± 0.21	19.19 ± 0.18 *

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at *p< 0.05 when compared with repaglinide alone

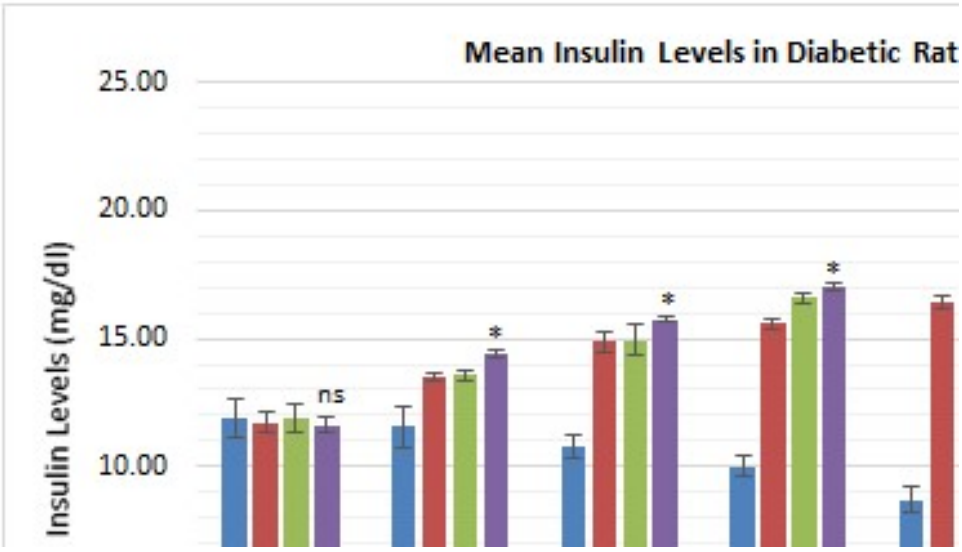


Figure 7.4. Insulin levels in Diabetic Rats.

ns- non significant, significant at *p< 0.05 when compared with repaglinide alone

7.2. PD Interactions Study of Repaglinide with Piperine in Rabbits

7.2.1. Repaglinide and Piperine Interactions in Normal Rabbits: A Study of Blood Glucose Levels

Table 7.7. Blood glucose levels in Normal Rabbits.

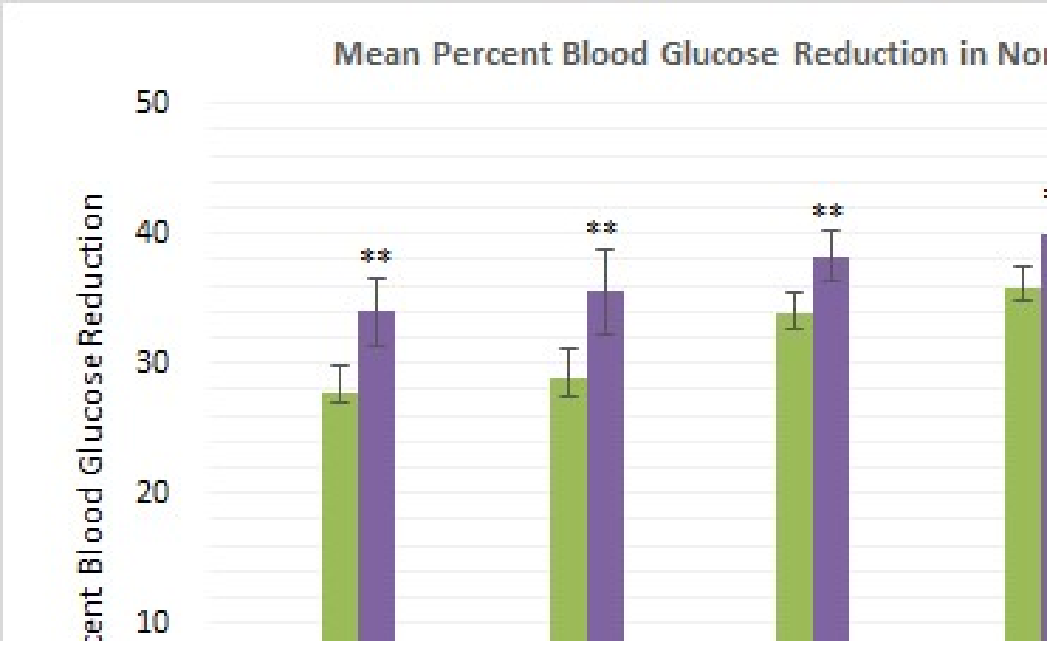
Type of Treatment	Blood Glucose Levels (mg/dl)				
	Day	**Vehicle**	**Piperine**	**Repaglinide**	**Piperine + Repaglinide**
	0	106.62 ± 1.44	107.92 ± 3.21	106.84 ± 2.73	107.32 ± 2.65 **ns**
SDT	**1**	106.22 ± 1.48	105.76 ± 3.48	77.11 ± 0.26	70.79 ± 2.18 **
MDT	**3**	107.79 ± 1.67	104.70 ± 3.20	75.93 ± 0.54	69.18 ± 2.74 **
	7	106.93 ± 0.67	102.92 ± 2.43	70.56 ± 0.60	66.19 ± 1.57 **
	14	105.71 ± 0.91	102.26 ± 2.31	68.51 ± 0.63	64.53 ± 1.22 **
	21	105.38 ± 0.84	101.52 ± 2.39	65.39 ± 0.71	61.12 ± 1.57 **

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at **p< 0.01 when compared with repaglinide alone

Table 7.8. Percent blood glucose reduction in Normal Rabbits.

Type of Treatment	Percent Blood Glucose Reduction (Mean ± SD)				
	Day	**Vehicle**	**Piperine**	**Repaglinide**	**Piperine + Repaglinide**
SDT	**1**	0.36 ± 1.58	2.00 ± 0.61	27.79 ± 1.96	34.01 ± 2.67 **
MDT	**3**	-1.12 ± 2.39	2.98 ± 0.54	28.89 ± 2.18	35.51 ± 3.22 **
	7	-0.30 ± 1.45	4.62 ± 0.64	33.93 ± 1.49	38.31 ± 1.99 **
	14	0.84 ± 0.55	5.23 ± 0.76	35.85 ± 1.67	39.86 ± 1.51 **
	21	1.15 ± 1.75	5.91 ± 0.67	38.78 ± 1.22	43.03 ± 1.99 **

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at **p< 0.01 when compared with repaglinide alone



ns- non significant, significant at **p< 0.01 when compared with repaglinide alone

Figure 7.5. Mean percent blood glucose reductions in Normal Rabbits.

7.2.2. Repaglinide and Piperine Interactions in Diabetic Rabbits: A Study of Blood Glucose Levels

Table 7.9. Blood glucose levels in Diabetic Rabbits

Type of Treatment	Blood Glucose Levels (mg/dl)				
	Day	Vehicle	Piperine	Repaglinide	Piperine + Repaglinide
	0	284.11 ± 1.94	284.85 ± 1.32	285.59 ± 3.93	284.56 ± 3.48 ns
SDT	**1**	303.00 ± 3.55	207.57 ± 1.47	201.30 ± 1.64	194.42 ± 2.44 **
MDT	**3**	324.12 ± 2.81	199.78 ± 1.11	193.51 ± 1.03	186.73 ± 3.16 **
	7	358.60 ± 5.71	193.6 ± 1.26	182.58 ± 1.35	172.32 ± 2.26 **
	14	371.03 ± 3.49	188.53 ± 0.93	174.43 ± 3.13	164.60 ± 2.44 **
	21	392.06 ± 4.68	182.28 ± 0.65	169.99 ± 3.26	158.88 ± 2.27 **

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at **p< 0.01 when compared with repaglinide alone

Table 7.10. Percent blood glucose reduction in Diabetic Rabbits.

Type of Treatment	Percent Blood Glucose Reduction (Mean ± SD)				
	Day	Vehicle	Piperine	Repaglinide	Piperine + Repaglinide
SDT	**1**	-6.65 ± 0.63	27.13 ± 0.57	29.50 ± 1.39	31.68 ± 0.62 *
MDT	**3**	-14.08 ± 0.82	29.87 ± 0.43	32.24 ± 0.87	34.38 ± 1.07 *
	7	-26.23 ± 2.45	32.03 ± 0.31	36.06 ± 1.30	39.44 ± 0.76 *
	14	-30.59 ± 1.05	33.81 ± 0.41	38.90 ± 1.91	42.15 ± 1.11 *
	21	-37.99 ± 0.94	36.01 ± 0.30	40.46 ± 1.94	44.16 ± 0.77 *

SDT- Single-dose treatment, MDT-Multiple-dose treatment
significant at *p< 0.05 when compared with repaglinide alone

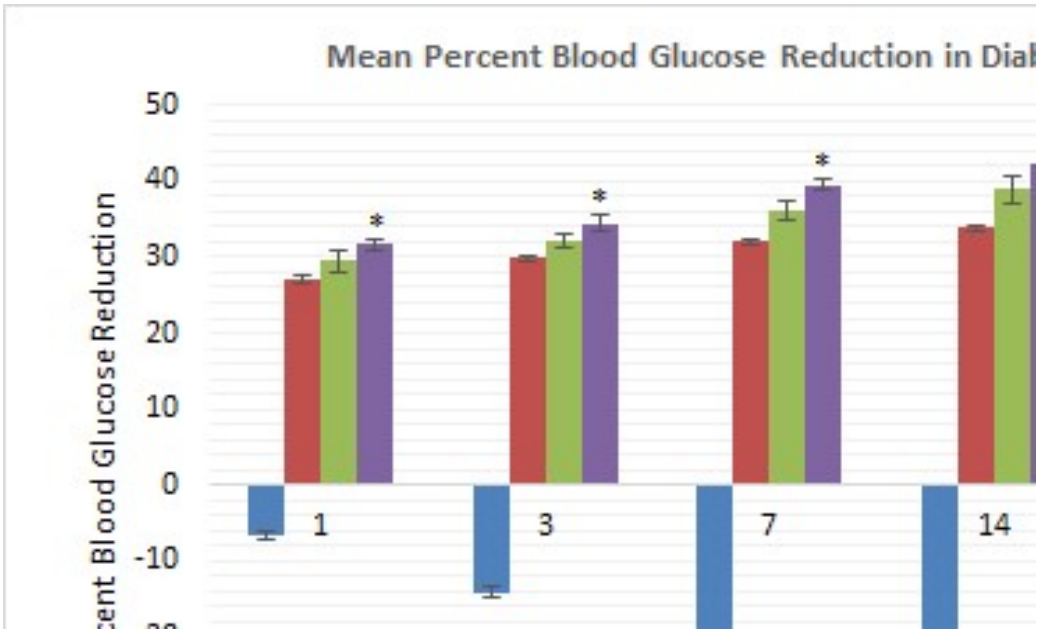


Figure 7.6. Mean percent blood glucose reduction in Diabetic Rabbits.

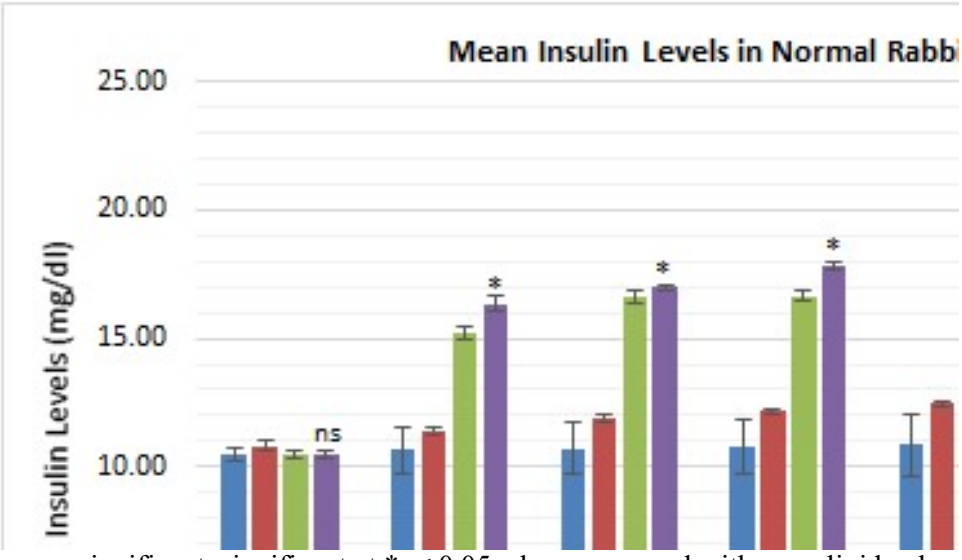
significant at *p< 0.05 when compared with repaglinide alone

7.2.3. Repaglinide and Piperine Interactions in Normal Rabbits: A Study of Insulin Levels

Table 7.11. Insulin levels in Normal Rabbits

Type of Treatment	Insulin Levels (mIU/L)				
	Day	Vehicle	Piperine	Repaglinide	Piperine + Repaglinide
	0	10.48 ± 0.21	10.80 ± 0.19	10.43 ± 0.15	10.47 ± 0.15 ns
SDT	**1**	10.63 ± 0.87	11.37 ± 0.12	15.21 ± 0.21	16.34 ± 0.28 *
MDT	**3**	10.72 ± 1.03	11.88 ± 0.17	16.64 ± 0.25	16.99 ± 0.09 *
	7	10.76 ± 1.04	12.15 ± 0.11	16.64 ± 0.19	17.80 ± 0.13 *
	14	10.83 ± 1.22	12.45 ± 0.14	17.42 ± 0.15	18.86 ± 0.14 *
	21	11.20 ± 1.42	12.94 ± 0.08	17.79 ± 0.22	19.40 ± 0.24 *

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at *p< 0.05 when compared with repaglinide alone



ns- non significant, significant at *p< 0.05 when compared with repaglinide alone

Figure 7.7. Insulin levels in Normal Rabbits.

7.2.4. Repaglinide and Piperine Interactions in Diabetic Rabbits: A Study of Insulin Levels

Table 7.12. Insulin levels in Diabetic Rabbits.

Type of Treatment	Insulin Levels (mIU/L)				
	Day	Vehicle	Piperine	Repaglinide	Piperine + Repaglinide
	0	10.67 ± 0.26	10.68 ± 0.37	10.74 ± 0.34	10.81 ± 0.36 ns
SDT	**1**	9.49 ± 0.29	15.51 ± 0.32	16.46 ± 0.18	17.06 ± 0.18 *
MDT	**3**	8.38 ± 0.20	16.24 ± 0.29	17.44 ± 0.41	18.29 ± 0.53 *
	7	8.64 ± 0.22	17.49 ± 0.61	18.83 ± 0.30	19.73 ± 0.26 *
	14	7.19 ± 0.10	18.82 ± 0.20	20.73 ± 0.18	21.12 ± 0.24 *
	21	7.30 ± 0.64	19.98 ± 0.54	21.51 ± 0.14	22.57 ± 0.15 *

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at *p< 0.05 when compared with repaglinide alone

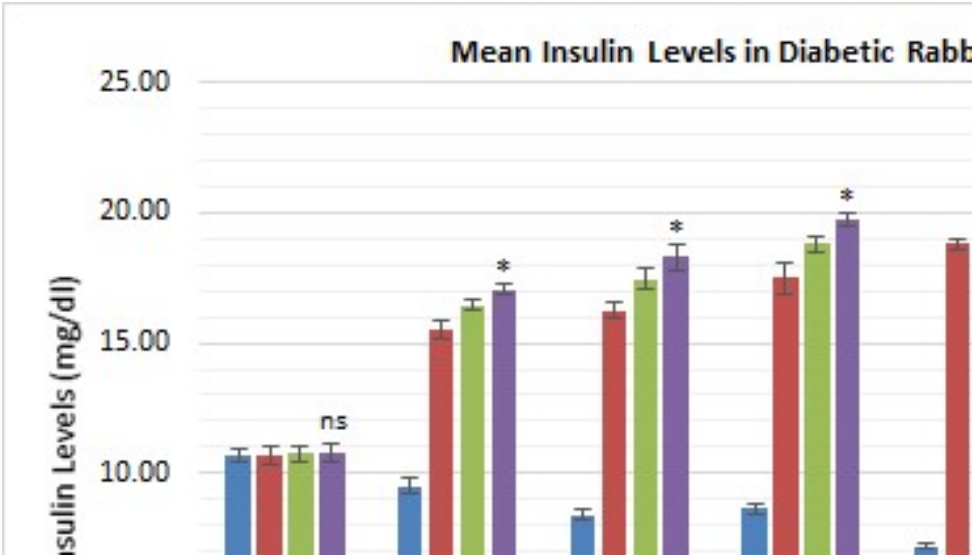


Figure 7.8. Insulin levels in Diabetic Rabbits.

ns- non significant, significant at *p< 0.05 when compared with repaglinide alone

7.3. Discussion for PDs of Repaglinide with Piperine

The term "PD interactions" implies interactions in which two drugs' effects are directly affected by one another [256]. HDIs are frequently categorized as synergistic, additive, or antagonistic. The interaction mechanism within a complicated pathophysiological system may occur at the same target or via different paths [257].

This study compared single and multi doses of Repaglinide to understand the impact of piperine in rats (normal and diabetic) and rabbits (normal and diabetic) so as to evaluate the PD interaction of Repaglinide with Piperine.

Piperine alone has no hypoglycemic action in normal rats and normal rabbits, resulting in a very little drop in glucose levels (tables 7.1 & 7.7), a very slight rise in percent blood glucose reduction (tables 7.2 & 7.8) and insulin levels (tables 7.5 & 7.11) on day 21 as compared to day 1. However, when diabetic rats and diabetic rabbits are treated with piperine alone, they demonstrate antihyperglycemic action by showing decreased blood glucose levels (tables 7.3 & 7.9) and higher insulin levels (tables 7.6 & 7.12) on day 21 as compared to day 1. Repaglinide alone treatment in normal rats and normal rabbits shows hypoglycemic action (tables 7.1 & 7.7) and in diabetic rats and diabetic rabbits shows anti-hyperglycemic activity (tables 7.3 & 7.9) on day 1 and day 21. Moreover, repaglinide alone treatment in both rats (normal and streptozotocin-induced diabetes) and rabbits (normal and streptozotocin-induced diabetes) showed elevated percent blood glucose reduction and raised insulin levels on day 1 and day 21 (tables 7.2, 7.4, 7.8 & 7.10). However, the results show that a multiple-dose study of piperine alone and repaglinide alone treatment is more effective in decreasing blood glucose levels (tables 7.1, 7.3, 7.7 & 7.9), increasing percent blood glucose reduction (tables 7.2, 7.4, 7.8 & 7.10) and increasing insulin levels (tables 7.5, 7.6, 7.11 & 7.12) as compared with single-dose study in rats (normal and streptozotocin-induced diabetes) and rabbits (normal and streptozotocin-induced diabetes).

Co-administration of repaglinide with piperine was shown to maintain hypoglycemic activity in normal animals (rats and rabbits) (tables 7.1 & 7.7) and anti-

hyperglycemic activity in diabetic animals (rats and rabbits) (tables 7.3 & 7.9) from day 1 to day 21 in this PD study.

The percent glucose reduction levels of single-dose treatment for combined therapy of repaglinide and piperine (on day 1) in normal rats and normal rabbits were observed to be 34.32 ± 7.68 and 34.01 ± 2.67, respectively, as compared with repaglinide alone therapy was observed to be 19.08 ± 11.67 and 27.79 ± 1.96 respectively (tables 7.2 & 7.8).

The percent glucose reduction levels of multiple-dose treatment for combined therapy of repaglinide and piperine (on day 21) in normal rats and rabbits were observed to be 47.50 ± 5.35 and 43.03 ± 1.99 respectively, as compared with repaglinide alone therapy was observed to be 40.51 ± 3.97 and 38.78 ± 1.22 respectively (table 7.3 & table 7.9).

The percent glucose reduction levels of single-dose treatment for combined therapy of repaglinide and piperine (on day 1) in diabetic rats and diabetic rabbits were observed to be 40.74 ± 3.68 and 31.68 ± 0.62, respectively, as compared with repaglinide alone therapy was observed to be 32.76 ± 3.61 and 29.50 ± 1.39 respectively (table 7.5 & 7.11).

The percent glucose reduction levels of multiple-dose treatment for combined therapy of repaglinide and piperine (on day 21) in diabetic rats and diabetic rabbits were observed to be 56.09 ± 1.66 and 44.16 ± 0.77, respectively, as compared with repaglinide alone therapy was observed to be 49.01 ± 3.94 and 40.46 ± 1.94 respectively (tables 7.5 & 7.11).

Repaglinide, along with piperine treatment in rats (normal and streptozotocin-induced diabetes) and rabbits (normal and streptozotocin-induced diabetes), resulted in significantly ($p<0.05$, $p<0.01$) decreased blood glucose levels (tables 7.2, 7.4, 7.8 & 7.10), significantly ($p<0.05$) augmented percent blood glucose reduction (tables 7.3, 7.5, 7.9 & 7.11) and significantly ($p<0.05$, $p<0.01$) increased insulin levels (tables 7.6, 7.7, 7.12 & 7.13) as compared with repaglinide alone therapy for both single and multiple-dose treatment. Conversely, the study results showed that multiple-dose

therapy was more effective than single-dose therapy in both normal animals (rats and rabbits) and diabetic animals (rats and rabbits).

Repaglinide reduces blood glucose levels by inducing the pancreatic β-cells to generate more insulin. Insulin synthesis is glucose-dependent, and it declines when glucose levels fall. Repaglinide binds to particular sites in the β-cell membrane and inhibits ATP-dependent potassium channels. The β-cell depolarizes due to the potassium channel blockage, forcing calcium channels to open. The increased calcium influx increases insulin secretion [172]. Repaglinide is quickly absorbed after oral administration and metabolized by cytochrome P450 3A4 and 2C8 through oxidation and de-alkylation [173].

Piperine is a thermonutrient herbal medicine that may help to increase nutrient absorption by increasing thermogenesis. In the gastrointestinal tract, the autonomous nervous system represents two main receptors, the α- and β-adrenergic receptors. [258], according to the concept of piperine-induced thermogenesis. Piperine has been demonstrated to exhibit β-agonistic action on adrenergic receptors, according to Majeed et al. in their piperine patent [259]. β-receptors, which contain a well-known substance, i.e. cyclic adenosine 3', 5' monophosphate (cAMP), aid in piperine-induced thermogenesis. The cAMP is an important "second messenger" in the body's hormonal and enzymatic activities. The need for new nutrients to maintain metabolic activities increases fast when thermogenesis occurs [258]. The activation of β3-receptors increases the proliferation of β-cells and will produce more insulin. Blood glucose levels drop due to the released insulin [260]. As a result, piperine's acute effects might be explained by a selective or relatively strong agonism at these receptors. As per recent studies, piperine inhibits the CYP P450 enzyme isoforms, i.e., CYP1A2, CYP1A1, CYP2D6, CYP3A4, and CYP2C8 selectively [261]. Piperine also affects the absorption of co-administered drugs in the intestine, resulting in drug interactions [262, 263].

When piperine was combined with repaglinide, the results showed that piperine significantly ($p<0.01$, $p<0.05$) raised percent glucose reduction (tables 7.3, 7.5, 7.9 & 7.11) and significantly ($p<0.05$) increased insulin levels (tables 7.6, 7.7, 7.12 & 7.13) synergistically as compared with piperine alone. Repaglinide has a rapid

start and a short duration of action, according to previous research [264]. According to Sama et al., Nateglinide and piperine influenced the β-cells to release more insulin in a synergistic manner [265]. According to the literature, repaglinide is a short-acting secretagogue that stimulates the pancreatic β-cells to emit insulin, while piperine activates the β-cells' β-receptors to increase insulin release. This study shows that piperine and repaglinide have a synergistic effect on β-cells, causing them to generate more insulin and, as a result, lowers the blood glucose levels, as seen by enhanced hypoglycemic action in normal rats and normal rabbits and enhanced antihyperglycemic action in diabetic rats and diabetic rabbits. In the current study, the hypoglycemia impact seen in normal animals (rats and rabbits) owing to a synergistic effect on β-cells' ability to release significantly ($p<0.05$) more insulin caused significantly ($p<0.0$) diminished the blood glucose levels when piperine and repaglinide are given together, while piperine alone has no effect. This study shows that piperine increases repaglinide's activity when used at the recommended dose.

Piperine may reduce blood glucose levels after co-administration of metformin due to improved gastrointestinal absorption, which is likely related to increased micelle production, gastrointestinal flow, and higher permeability due to epithelial cell alteration [266]. Piperine also lowered nateglinide blood glucose levels, most likely related to metabolic inhibition of the CYP 3A4 and 2C9 enzymes [265, 203]. Following the review of literatures, the study findings also revealed that piperine enhanced gastrointestinal absorption when treated along with repaglinide and inhibited the CYP 3A4 isoform enzymes of repaglinide via the efflux mechanism of repaglinide. This study demonstrates that piperine impacted repaglinide absorption and metabolism in the intestine, resulting in a piperine-repaglinide drug interaction.

Conclusion

1. The results of this investigation showed the presence of PD interaction between Repaglinide and Piperine in normal as well as diabetic animals (rats and rabbits).

2. The study findings showed significant ($p<0.05$, $p<0.01$) anti hyperglycemic action in diabetic animals (rats and rabbits) and significant ($p<0.05$, $p<0.01$) hypoglycemic

impact in normal animals (rats and rabbits) when treated with piperine plus repaglinide as compared with alone repaglinide.

3. Study findings in diabetic animal models of two dissimilar species, indicate the possibility of similar interaction in humans as well, necessitating dose adjustments. Caution should be used when this combination is suggested for therapeutic benefit.

7.4. PK Interaction study of Repaglinide with Piperine in Rabbits

7.4.1 PK Interaction study of Repaglinide with Piperine in Normal Rabbits

Table 7.13. Mean Plasma Concentrations of Repaglinide in Normal Rabbits after treatment with Repaglinide in the absence and presence of Piperine on each Day 1 and Day 21.

Mean Plasma Concentration of Repaglinide (ng/ml) in Normal Rabbits				
Time/Hr	**Day 1 (SDT)**		**Day 21 (MDT)**	
	Repaglinide	**Piperine + Repaglinide**	**Repaglinide**	**Piperine + Repaglinide**
0	0 ± 0.00	0 ± 0	0 ± 0	0 ± 0
0.25	9.92 ± 0.81	12.31 ± 0.23	11.08 ± 0.86	13.34 ± 0.79
0.5	29.26 ± 1.51	32.98 ± 1.11	30.49 ± 1.39	33.17 ± 1.11
1	34.68 ± 1.10	36.57 ± 0.59	34.56 ± 1.22	37.02 ± 1.23
1.5	20.74 ± 1.62	32.02 ± 1.24	22.35 ± 0.94	30.56 ± 1.82
2	18.31 ± 0.54	21.53 ± 0.74	19.73 ± 1.32	22.16 ± 1.26
3	13.53 ± 0.55	16.58 ± 0.52	15.38 ± 0.73	17.12 ± 0.38
4	9.84 ± 0.66	11.38 ± 0.73	10.88 ± 0.37	13.16 ± 0.57
6	2.58 ± 1.01	8.03 ± 0.86	3.01 ± 1.03	7.79 ± 0.43
8	0 ± 0.00	2.93 ± 1.02	0 ± 0	3.21 ± 1.01

SDT- Single-dose treatment, MDT-Multiple-dose treatment

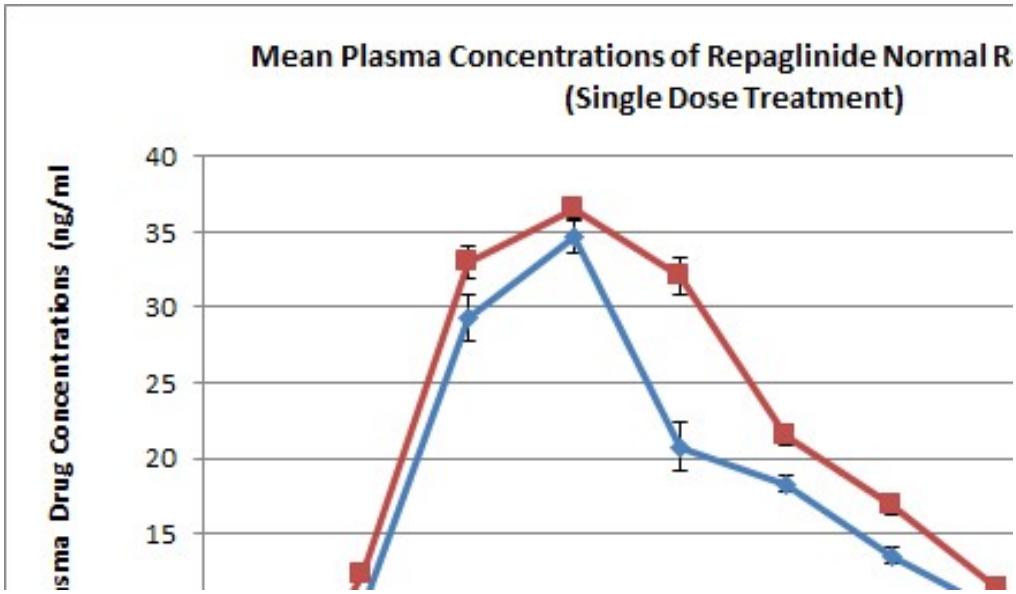


Figure 7.9. Mean Plasma Concentrations of Repaglinide in Normal Rabbits after treatment with Repaglinide in the absence and presence of Piperine on each Day 1

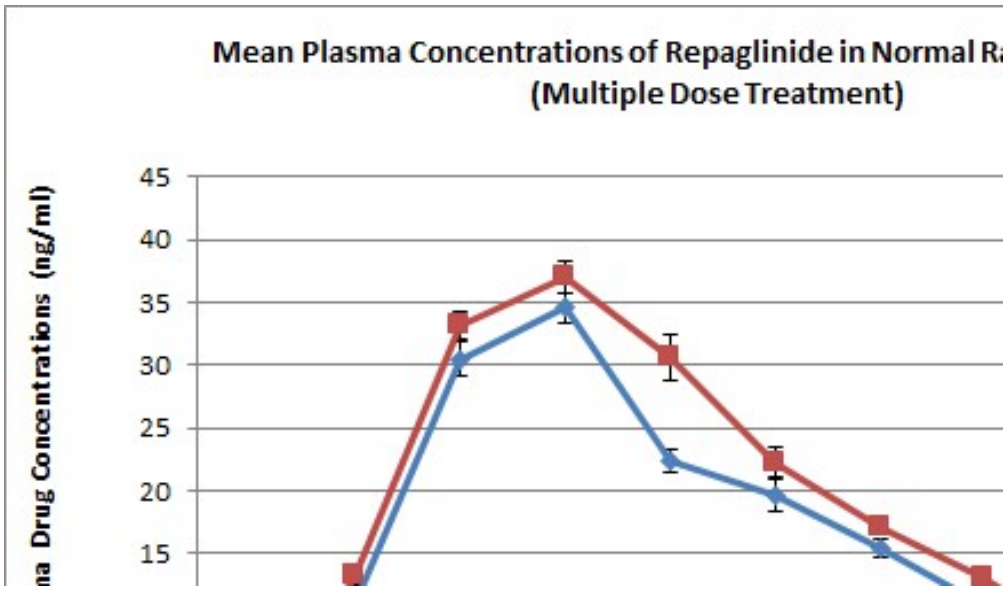


Figure 7.10. Mean Plasma Concentrations of Repaglinide in Normal Rabbits after treatment with Repaglinide in the absence and presence of Piperine on Day 21

Table 7.14. Mean PK Parameters of Repaglinide in Normal Rabbits after treatment with Repaglinide in the absence and presence of Piperine on each Day 1 and Day 21

PK Parameters	Day 1 (SDT)		Day 21 (MDT)	
	Repaglinide	Piperine + Repaglinide	Repaglinide	Piperine + Repaglinide
C_{max} (ng/ml)	34.68 ± 1.10	36.57 ± 0.59 *	34.56 ± 1.22	37.02 ± 1.23 *
T_{max} (h)	1 ± 0	1 ± 0.00 ns	1 ± 0.00	1 ± 0.00 ns
AUC_{0-t} (ng/ml*h)	85.75 ± 1.35	118.78 ± 4.04 ***	92.15 ± 0.35	121.83 ± 2.78 ***
$AUC_{0-\infty}$ (ng/ml*h)	91.07 ± 4.39	128.13 ± 8.06 ***	98.15 ± 3.75	132.12 ± 5.85 ***
$AUMC_{0-t}$ (ng/ml*h^2)	181.52 ± 7.52	320.1 ± 22.40 ***	199.45 ± 6.49	331.22 ± 13.02 ***
$AUMC_{0-\infty}$ (ng/ml*h^2)	224.99 ± 37.51	425.38 ± 76.71 **	248 ± 36.64	446.89 ± 62.99 **
MRT_{0-t} (h)	2.12 ± 0.06	2.69 ± 0.10 ***	2.16 ± 0.06	2.72 ± 0.06 ***
$MRT_{0-\infty}$ (h)	2.46 ± 0.30	3.3 ± 0.40 *	2.53 ± 0.28	3.37 ± 0.35 **
K_e (1/h)	0.57 ± 0.18	0.34 ± 0.06 *	0.56 ± 0.14	0.33 ± 0.05 *
$T_{1/2}$ (h)	1.31 ± 0.41	2.11 ± 0.39 *	1.3 ± 0.35	2.15 ± 0.32 *
CL (mg/kg)/(ng/ml)/h	0.0033 ± 0.0002	0.0023 ± 0.0002 ***	0.0031 ± 0.0001	0.0023 ± 0.0001 ***
Vd (L)	0.0062 ± 0.0016	0.0071 ± 0.0009 ns	0.0057 ± 0.0013	0.0070 ± 0.0008 ns

SDT- Single-dose treatment, MDT-Multiple-dose treatment

ns- non significant, significant at *p< 0.05, **p< 0.01, ***p< 0.001 when compared with repaglinide alone

Table 7.15. Mean PK Parameters of Repaglinide with piperine in Normal Rabbits on Day 1 and Day 21

PK Parameters	Day 1 (SDT)	Day 21 (MDT)
	Piperine + Repaglinide	Piperine + Repaglinide
C_{max} (ng/ml)	36.57 ± 0.59	37.02 ± 1.23 *
T_{max} (h)	1 ± 0.00 ns	1 ± 0.00 ns
AUC_{0-t} (ng/ml*h)	118.78 ± 4.04	121.83 ± 2.78 ***
$AUC_{0-\infty}$ (ng/ml*h)	128.13 ± 8.06	132.12 ± 5.85 ***
$AUMC_{0-t}$ (ng/ml*h^2)	320.1 ± 22.40	331.22 ± 13.02 ***
$AUMC_{0-\infty}$ (ng/ml*h^2)	425.38 ± 76.71	446.89 ± 62.99 **
MRT_{0-t} (h)	2.69 ± 0.10	2.72 ± 0.06 ***
$MRT_{0-\infty}$ (h)	3.3 ± 0.40	3.37 ± 0.35 *
K_e (1/h)	0.34 ± 0.06	0.33 ± 0.05 *
$T_{1/2}$ (h)	2.11 ± 0.39	2.15 ± 0.32 *
CL (mg/kg)/(ng/ml)/h	0.0023 ± 0.0002	0.0023 ± 0.0001 ***
Vd (L)	0.0071 ± 0.0009	0.0070 ± 0.0008 ns

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at *p< 0.05, **p< 0.01, ***p< 0.001 when compared with day 1

7.4.2 PK Interactions study of Repaglinide with Piperine in Diabetic Rabbits

Table 7.16. Mean Plasma Drug Concentrations of Repaglinide in Diabetic Rabbits after treatment with Repaglinide in the absence and presence of Piperine on each Day 1 and Day 21

Mean Plasma Drug Concentrations of Repaglinide (ng/ml) in Diabetic Rabbits				
Time/Hr	**Day 1 (SDT)**		**Day 21 (MDT)**	
	Repaglinide	**Piperine + Repaglinide**	**Repaglinide**	**Piperine + Repaglinide**
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.25	10.08 ± 0.46	11.24 ± 0.73	11.09 ± 0.59	12.46 ± 0.78
0.5	29.63 ± 1.42	32.51 ± 1.00	32.38 ± 1.01	34.52 ± 1.08
1	35.28 ± 1.75	37.64 ± 0.49	35.87 ± 1.27	38.29 ± 1.15
1.5	22.75 ± 1.67	29.06 ± 1.41	25.40 ± 1.50	30.09 ± 1.30
2	18.52 ± 0.73	21.09± 1.09	19.05 ± 1.26	22.99 ± 1.31
3	14.04 ± 0.45	15.97 ± 0.57	17.18 ± 0.67	19.35 ± 1.05
4	10.14 ± 0.46	11.47 ± 0.56	11.05 ± 0.71	13.71 ± 0.92
6	3.44 ± 1.04	8.52 ± 1.04	4.32 ± 1.59	9.78 ± 1.11
8	0 ± 0	3.28 ± 0.95	0 ± 0	3.49 ± 1.04

SDT- Single-dose treatment, MDT-Multiple-dose treatment

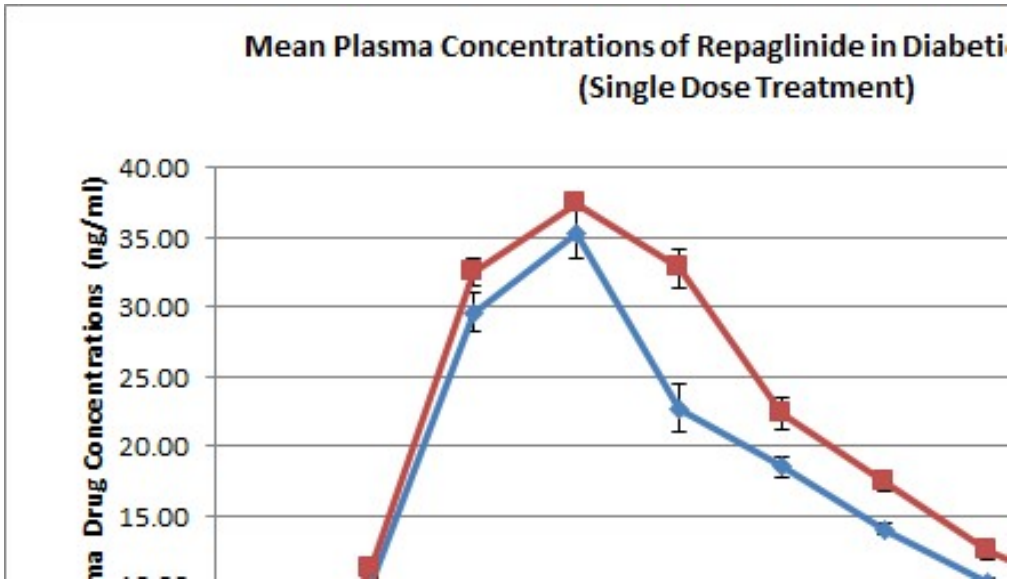


Figure 7.11. Mean Plasma Drug Concentrations of Repaglinide in Diabetic Rabbits after treatment with Repaglinide in the absence and presence of Piperine on Day 1

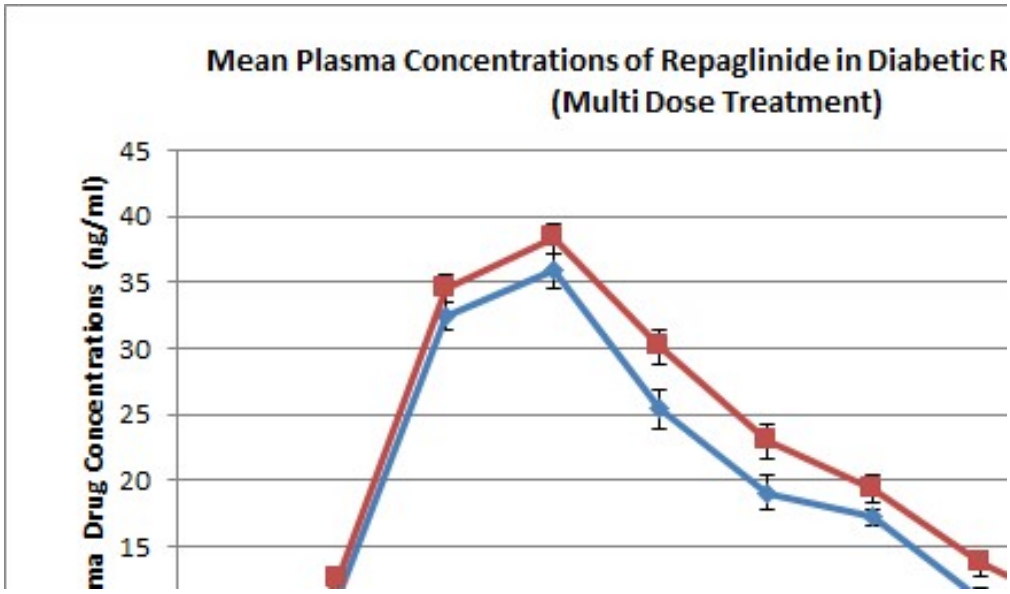


Figure 7.12. Mean Plasma Drug Concentrations of Repaglinide in Diabetic Rabbits after treatment with Repaglinide in the absence and presence of Piperine on Day 21

Table 7.17. Mean PK Parameters of Repaglinide in Diabetic Rabbits after treatment with Repaglinide in the absence and presence of Piperine on each Day 1 and Day 21

PK Parameters	Day 1 (SDT)		Day 21 (MDT)	
	Repaglinide	Piperine + Repaglinide	Repaglinide	Piperine + Repaglinide
C_{max} (ng/ml)	35.28 ± 1.75	37.64 ± 0.49 *	35.87 ± 1.27	38.29 ± 1.15 *
T_{max} (h)	1 ± 0.00	1 ± 0.00 ns	1 ± 0.00	1 ± 0.00 ns
AUC_{0-t} (ng/ml*h)	89.22 ± 1.31	117.65 ± 3.55 ****	97.91 ± 3.15	130.43 ± 4.49 ****
$AUC_{0-\infty}$ (ng/ml*h)	97.5 ± 3.98	129.1 ± 3.99 ****	108.14 ± 8.58	142.00 ± 8.82 **
$AUMC_{0-t}$ (ng/ml*h^2)	192.25 ± 6.38	324.17 ± 14.80 ****	216.1 ± 14.08	369.06 ± 24.81 ****
$AUMC_{0-\infty}$ (ng/ml*h^2)	262.09 ± 37.75	456.8 ± 69.27 **	302.63 ± 67.90	500.09 ± 82.02 **
MRT_{0-t} (h)	2.15 ± 0.07	2.75 ± 0.07 **	2.21 ± 0.08	2.83 ± 0.10 ****
$MRT_{0-\infty}$ (h)	2.68 ± 0.28	3.53 ± 0.43 *	2.77 ± 0.41	3.51 ± 0.36 *
K_e (1/h)	0.43 ± 0.06	0.3 ± 0.05 *	0.48 ± 0.13	0.31 ± 0.04 ns
$T_{1/2}$ (h)	1.62 ± 0.22	2.33 ± 0.42 *	1.53 ± 0.41	2.23 ± 0.29 *
CL (mg/kg)/(ng/ml)/h	0.0031 ± 0.0001	0.0023 ± 0.0002 ****	0.0028 ± 0.0002	0.0021 ± 0.0001 **
Vd (L)	0.0072 ± 0.0007	0.0078 ± 0.0012 ns	0.006 ± 0.0012	0.0068 ± 0.0005 ns

SDT- Single-dose treatment, MDT-Multiple-dose treatment

ns- non significant, significant at *p< 0.05, **p< 0.01, ***p< 0.001 when compared with repaglinide alone

Table 7.18. Mean PK Parameters of Repaglinide with piperine in Diabetic Rabbits on Day 1 and Day 21

PK Parameters	Day 1 (SDT)	Day 21 (MDT)
	Piperine + Repaglinide	Piperine + Repaglinide
C_{max} (ng/ml)	37.64 ± 0.49	38.29 ± 1.15 ns
T_{max} (h)	1 ± 0.00 ns	1 ± 0.00 ns
AUC_{0-t} (ng/ml*h)	117.65 ± 3.55	130.43 ± 4.49 **
$AUC_{0-\infty}$ (ng/ml*h)	129.1 ± 3.99	142.00 ± 8.82 *
$AUMC_{0-t}$ (ng/ml*h^2)	324.17 ± 14.80	369.06 ± 24.81 *
$AUMC_{0-\infty}$ (ng/ml*h^2)	456.8 ± 69.27	500.09 ± 82.02 ns
MRT_{0-t} (h)	2.75 ± 0.07	2.83 ± 0.10 ns
$MRT_{0-\infty}$ (h)	3.53 ± 0.43	3.51 ± 0.36 ns
K_e (1/h)	0.3 ± 0.05	0.31 ± 0.04 ns
$T_{1/2}$ (h)	2.33 ± 0.42	2.23 ± 0.29 ns
CL (mg/kg)/(ng/ml)/h	0.0023 ± 0.0002	0.0021 ± 0.0001 ns
Vd (L)	0.0078 ± 0.0012	0.0068 ± 0.0005 ns

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at *p< 0.05, **p< 0.01 when compared with day 1

7.5. Discussion

The study's findings might be attributable to piperine's bio-enhancing properties when combined with repaglinide (tables 7.14 & 7.17). A "bio-enhancer" is a herbal substance (such as piperine) capable of increasing the bioefficacy and bioavailability of a drug when added to it despite having no conventional pharmacological action at the dose used [267]. HDI is mostly caused by physiologic systems' biotransformational pathways that relate substrate specificity to them [192], which can be a crucial step in optimizing the use of piperine with repaglinide, particularly in the management of chronic illnesses, such as DM.

This study investigated the PK interaction of repaglinide with piperine in rabbits (normal and streptozotocin-induced diabetes), comparing single and multiple repaglinide doses to see how piperine affected the result. There is evidence that piperine increases the bioavailability of various medications. In normal and diabetic rabbits, variations in repaglinide bioavailability in the presence of piperine were investigated.

In normal rabbits

The plasma concentration-time profile of repaglinide (figures 7.9 & 7.10) after per oral administration in the absence and presence of piperine was found to be significantly ($p<0.05$) different in normal rabbits on day 1 and day 21 and is presented in table 5.71. The PK parameters of repaglinide in the absence and presence of piperine in normal rabbits on day 1 and day 21 are summarized in table 7.14.

On day 1, the C_{max} of repaglinide, i.e., 34.68 ± 1.10 ng/ml was obtained in the absence of piperine therapy, and 36.57 ± 0.59 was obtained within the presence of piperine in normal rabbits (table 7.14). This study represents a significant ($p<0.05$) rise of 5.44% in peak plasma levels of repaglinide. Other PK parameters for comparing the bioavailability of repaglinide in the absence and presence of piperine are given in table 7.15. An increased C_{max} reduced clearance and increased $T_{1/2}$ contributed to an apparent increase in AUC. The results reveal that piperine significantly improved repaglinide bioavailability in a single-dose study.

On day 21, the C_{max} of repaglinide, i.e., 34.56 ± 1.22 ng/ml was obtained in the absence of piperine, and 37.02 ± 1.23 ng/ml was obtained within the presence of piperine in normal rabbits (table 7.14). This study represents a significant ($p<0.05$) increase of 7.11% in peak plasma levels of repaglinide. Other PK parameters by comparing the bioavailability of repaglinide in the absence and presence of piperine are given in table 7.15. An increased Cmax reduced clearance and increased $T_{1/2}$ contributed to an apparent increase in AUC. The above findings show that piperine significantly enriched the bioavailability of repaglinide in a multiple-dose study.

In diabetic rabbits

The plasma concentration-time report of repaglinide (figures 7.11 & 7.12) after oral administration of repaglinide in the absence and presence of was found to be significant ($p<0.05$) difference in diabetic rabbits on day 1 and day 21 and are presented in table 5.81. The PK parameters of repaglinide in the absence and presence of piperine in diabetic rabbits on day 1 and day 21 are summarized in table 7.17.

On day 1, the C_{max} of repaglinide, i.e., 35.28 ± 1.75 ng/ml was obtained in the absence of piperine, and 37.64 ± 0.49 ng/ml was obtained in the presence of piperine in diabetic rabbits (table 7.17). This study represents a significant ($p<0.05$) rise of 6.68% in peak plasma levels of repaglinide. Other PK parameters by comparing the bioavailability of repaglinide in the absence and presence of piperine are given in table 7.17. An increased Cmax reduced clearance and increased $T_{1/2}$ contributed to an apparent increase in AUC. The above findings show that piperine significantly raised the bioavailability of repaglinide in a single-dose study.

On day 21, the C_{max} of repaglinide was 35.87 ± 1.27 ng/ml was obtained in the absence of piperine, and 38.29 ± 1.15 ng/ml was obtained in the presence of piperine in diabetic rabbits (table 7.17). This study represents a significant ($p<0.05$) increase of 6.74% in peak plasma levels of Repaglinide. Other PK parameters by comparing the bioavailability of repaglinide in the absence and presence of piperine are given in table 7.18. An increased C_{max} reduced clearance and increased $T_{1/2}$ contributed to an

apparent increase in AUC. The above findings show that piperine significantly raised the bioavailability of repaglinide in a multiple-dose study.

The PK results in normal rabbits (day 1 & 21) (table 7.14) and diabetic rabbits (day1 & 21) (table 7.17) clearly show that piperine has not influenced the onset of action (T_{max}) of repaglinide but has significantly ($p<0.001$, $p<0.0001$) augmented total plasma exposure (AUC and AUMC). Because of the increased gastrointestinal absorption, there might be an interaction. Increased absorption can be attributed to (a) improved micelle formation, which leads to increased solubility, (b) improved gastrointestinal blood flow, (c) augmented permeability due to epithelial cell alteration, and (d) increased brush border membrane fluidity, which leads to increased microvilli length [268]. Piperine has been reported to enhance bioavailability by 30 to 200 percent [269], and repaglinide bioavailability is over 56 percent [173], with no change in repaglinide T_{max}. Repaglinide has a high protein binding of drugs (e.g., to 1-acid glycoprotein and albumin) of >98% [173], and Raghavendra K et al. show that piperine dislocates plasma-bound medications from 1-acid glycoprotein and albumin and promotes drug absorption through biological sheaths [270]. As a result, piperine has the potential to displace repaglinide from protein binding sites, potentially leading to higher levels of free repaglinide. However, the Vd of repaglinide was unaffected, indicating that the interaction does not involve protein displacement. As a result, decreased clearance and K_{el}, as well as increased MRT and $T_{1/2}$ (tables 7.14 & 7.17), indicate that the rise in repaglinide concentration levels in the presence of piperine may be due to a metabolic or excretion process.

The CYP system, particularly CYP3A4 and 2C8, converts repaglinide to inactive metabolites by oxidation and de-alkylation [173]. Piperine partially inhibits CYP 3A4 and CYP 2C8 in in-vivo studies, increasing the plasma/serum concentration levels of several drugs, such as azithromycin and carbamazepine [220, 237]. Piperine inhibits enzymes, which has major therapeutic consequences when drug metabolism is reduced, resulting in higher drug concentration and repaglinide bioavailability. Piperine's structure is responsible for its ability to block enzymes. The methylenedioxyphenyl (MDP) ring, piperidine moiety and side chain all work together to block 7-methoxycoumarin-Odemethylase (MOCD) and aryl hydrocarbon

hydroxylase (AHH) activities. Alteration of just one component in the piperine particle can cause differential inhibition of the two kinds of monooxygenase activity [268].

Repaglinide is predominantly removed 90% via feces (2% as an unaltered drug) and 8% (0.1 percent as an unchanged drug) via urine [173]. According to PK results like reduced CL & Ke and increased MRT & $T_{1/2}$, piperine causes time-dependent suppression of repaglinide metabolism, which results in delayed clearance and greater repaglinide plasma concentrations (tables 7.14 & 7.17). Other preclinical studies [213, 220, 223] have reported this metabolic inhibition.

In this investigation, the effects of multiple doses of repaglinide on piperine activity (plasma concentration level and PKs) were shown to be significantly higher than in single-dose therapy.

Conclusion

1. The study concludes that piperine increased the bioavailability of repaglinide.
2. Since the interaction was shown in rabbits, it is probable to happen in humans as well, leading to significantly greater repaglinide activity, which could prompt for dosage modifications. As a result, when this combination is recommended for therapeutic benefit, caution should be exercised.
3. Inhibition of CYP3A4 may be responsible for PK interaction between repaglinide and piperine.

8. SITAGLIPTIN WITH PIPERINE INTERACTION STUDY

8.1 PD interactions study of Sitagliptin with Piperine in Rats

8.1.1. PD interactions in Normal Rats (Effect on Blood glucose levels)

Table 8.1. Blood glucose levels in Normal Rats.

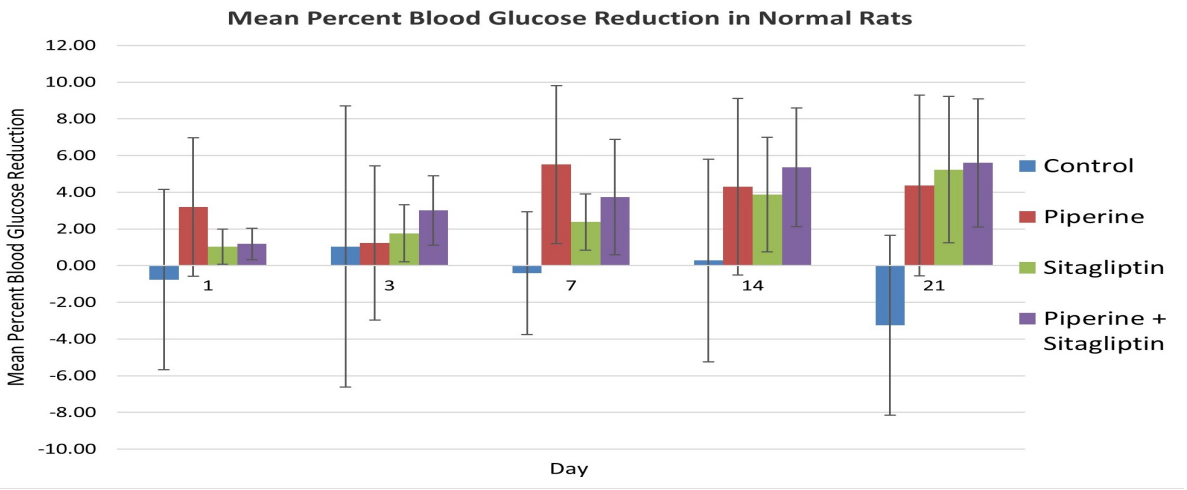
Type of Treatment	Blood Glucose Levels (mg/dl)				
	Day	Vehicle	Piperine	Sitagliptin	Piperine + Sitagliptin
	0	82.00 ±5.48	85.83 ± 4.22	88.17 ± 2.10	88.80 ± 2.02
SDT	**1**	81.33 ± 6.06	83.00 ± 2.97	87.26 ± 1.64	87.74 ± 1.45
MDT	**3**	83.00 ± 9.84	84.67± 3.01	86.62 ± 2.30	86.11 ± 2.09
	7	81.67 ± 6.12	81.00± 3.22	86.07 ± 1.90	85.44 ± 1.95
	14	82.00 ± 2.19	82.17± 6.01	84.73 ± 2.11	84.00 ± 2.05
	21	79.17 ± 3.60	82.00± 4.34	83.52 ± 2.67	83.79 ± 2.47

SDT- Single-dose treatment, MDT-Multiple-dose treatment
All values are expressed as mean ± SD (n=6). *p < 0.05; **p < 0.01 and ***p < 0.001.[a] when compared with vehicle control group, [b]when compared with Piperine alone group, [c] when compared with Repaglinide alone group.

Table 8.2. Percent blood glucose reductions in Normal Rats.

Type of Treatment	Percent Blood Glucose Reduction (Mean ± SD)				
	Day	Vehicle	Piperine	Sitagliptin	Piperine + Sitagliptin
SDT	**1**	-0.76 ±4.91	3.19 ±3.77	1.03 ± 0.96	1.18 ± 0.85
MDT	**3**	1.04 ± 7.67	1.23 ± 4.20	1.76 ± 1.56	3.01 ± 1.89
	7	-0.41 ±3.34	5.51 ± 4.31	2.38 ± 1.53	3.74 ± 3.14
	14	0.28 ± 5.52	4.29 ± 4.81	3.87 ± 3.13	5.36 ± 3.23
	21	-3.25 ± 4.91	4.37 ± 4.91	5.23 ± 3.99	5.6 ± 3.49

SDT- Single-dose treatment, MDT-Multiple-dose treatment
All values are expressed as mean ± SD (n=6). *p < 0.05; **p < 0.01 and ***p < 0.001.[a] when compared with vehicle control group, [b]when compared with Piperine alone group, [c] when compared with Repaglinide alone group.



ns-non significant when compared with sitagliptin alone

Figure 8.1. Mean percent blood glucose reduction in Normal Rats.

8.1.2. PD interactions in Diabetic Rats (Effect on Blood glucose levels)

Table 8.3. Blood glucose levels in Diabetic Rats.

Type of Treatment	Blood Glucose Levels (mg/dl)				
	Day	**Vehicle**	**Piperine**	**Sitagliptin**	**Piperine + Sitagliptin**
	0	311.26± 6.76	312.73 ± 7.95	309.14 ± 3.44 [b,***]	310.41 ± 3.63 [c,*]
SDT	**1**	317.50± 9.33	220.23 ± 12.97	182.67 ± 2.04 [b,***]	168.15 ± 4.39 [c,*]
MDT	**3**	325.88± 10.18	208.43 ± 7.56	175.15 ± 2.81 [b,***]	162.90 ± 7.38 [c,*]
	7	333.43± 9.08	195.83 ± 10.21	161.80 ± 2.09 [b,***]	147.79 ± 10.15 [c,*]
	14	340.03 12.56	187.50 ± 12.14	154.56 ± 2.41 [b,***]	139.45 ± 5.33 [c,*]
	21	347.46± 11.12	173.83 ±13.03	147.83 ± 2.18 [b,***]	131.90 ± 6.53 [c,*]

SDT- Single-dose treatment, MDT-Multiple-dose treatment
All values are expressed as mean ± SD (n=6). *p < 0.05; **p < 0.01 and ***p < 0.001.[a] when compared with vehicle control group, [b]when compared with Piperine alone group, [c] when compared with Repaglinide alone group.

Table 8.4. Percent blood glucose reduction in Diabetic Rats.

Type of Treatment	Percent Blood Glucose Reduction (Mean ± SD)				
	Day	Vehicle	Piperine	Sitagliptin	Piperine + Sitagliptin
SDT	1	-2.00 ± 1.89	29.59 ± 3.62	40.90 ± 1.01 ^{b,***}	45.82 ± 1.84 ^{c,*}
MDT	3	-4.71 ± 2.77	33.31 ± 2.96	43.34 ± 0.97 ^{b,***}	47.50 ± 2.85 ^{c,*}
	7	-7.15 ± 2.91	37.34 ± 3.72	47.66 ± 0.73 ^{b,***}	52.39 ± 3.22 ^{c,*}
	14	-9.23 ± 2.66	40.03 ± 3.89	50.00 ± 0.99 ^{b,***}	55.07 ± 1.81 ^{c,*}
	21	-11.64 ± 2.178	44.38 ± 4.46	52.18 ± 0.53 ^{b,*}	57.50 ± 2.14 ^{c,***}

SDT- Single-dose treatment, MDT-Multiple-dose treatment
All values are expressed as mean ± SD (n=6). *p < 0.05; **p < 0.01 and ***p < 0.001.[a] when compared with vehicle control group, [b]when compared with Piperine alone group, [c] when compared with Repaglinide alone group.

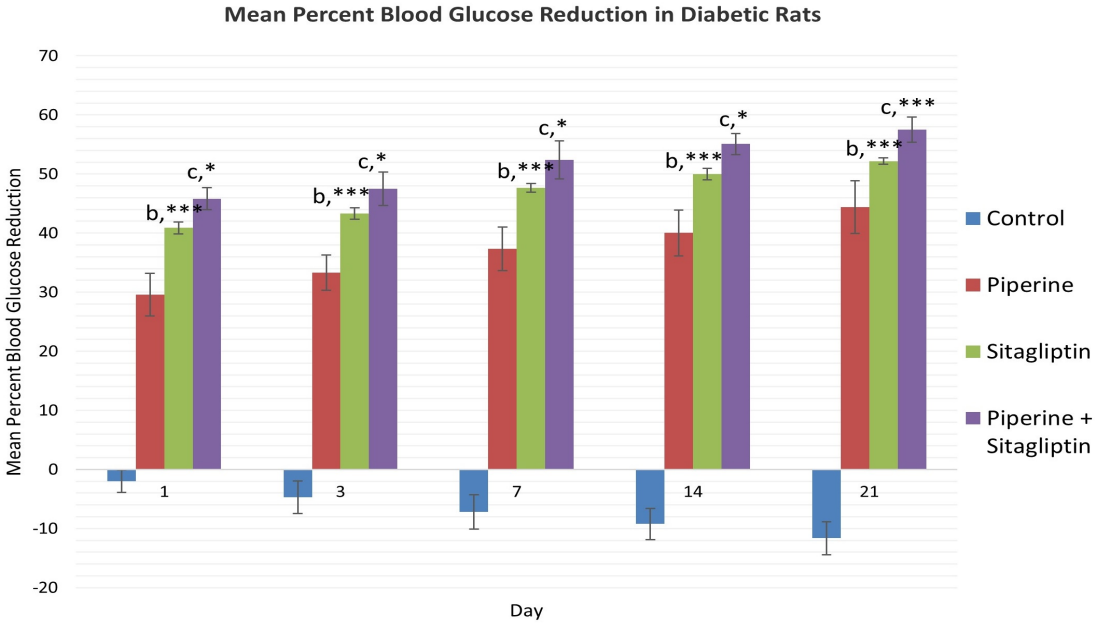


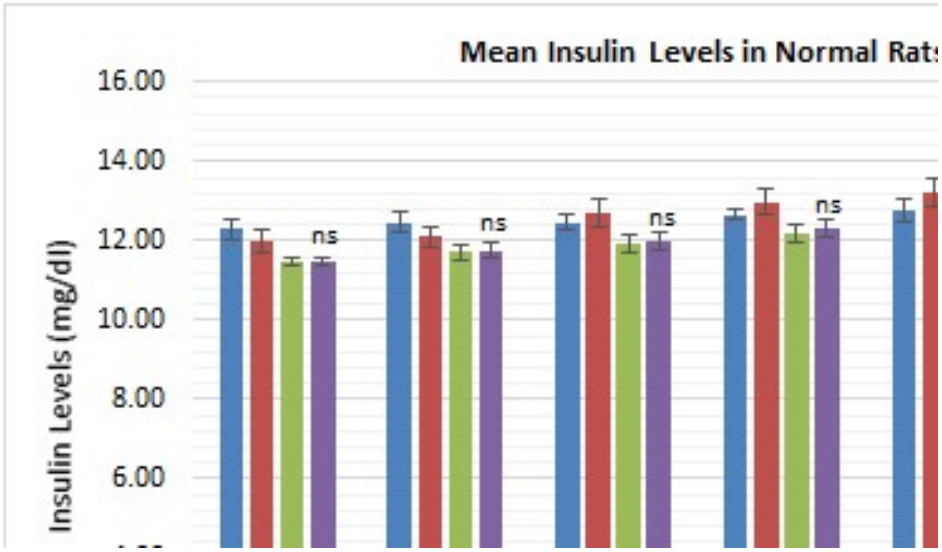
Figure 8.2. Mean percent blood glucose reduction in Diabetic Rats.

8.1.3. Sitagliptin and Piperine Interactions in Normal Rats: A Study of Insulin Levels

Table 8.5. Insulin levels in Normal Rats.

Type of Treatment	Insulin Levels (mIU/L)				
	Day	Vehicle	Piperine	Sitagliptin	Piperine + Sitagliptin
SDT	0	12.29 ± 0.25	11.97 ± 0.30	11.47 ± 0.09	11.46 ± 0.11 ns
	1	12.46 ± 0.25	12.09 ± 0.27	11.70 ± 0.20	11.76 ± 0.20 ns
MDT	3	12.46 ± 0.18	12.69 ± 0.37	11.94 ± 0.24	12.01 ± 0.23 ns
	7	12.65 ± 0.14	12.98 ± 0.32	12.17 ± 0.22	12.30 ± 0.24 ns
	14	12.76 ± 0.27	13.22 ± 0.38	12.27 ± 0.12	12.51 ± 0.25 ns
	21	12.29 ± 0.28	13.79 ± 0.42	12.54 ± 0.13	12.70 ± 0.21 ns

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non-significant when compared with sitagliptin alone



ns- non-significant when compared with sitagliptin alone

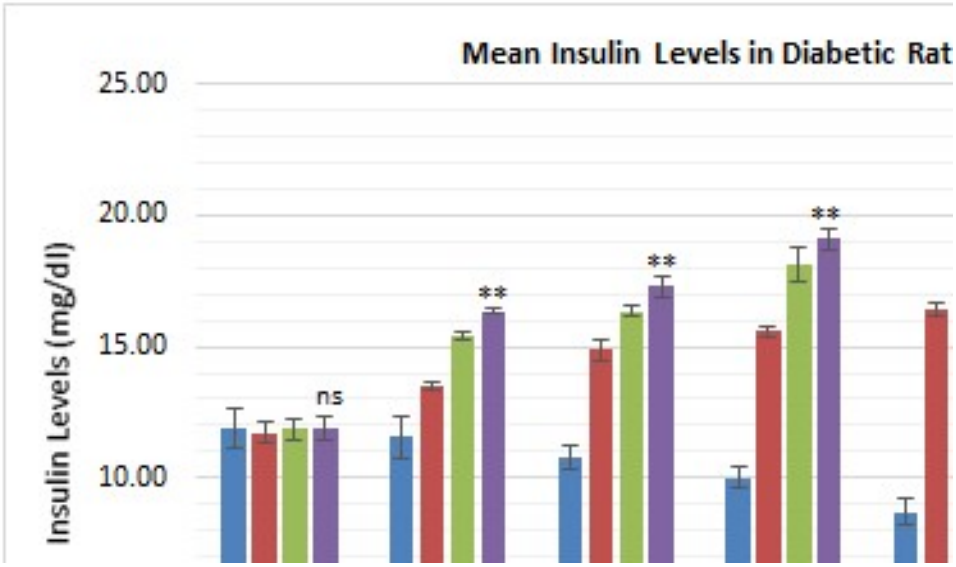
Figure 8.3. Insulin levels in Normal Rats.

8.1.4. Sitagliptin and Piperine Interactions in Diabetic Rats: A Study of Insulin Levels

Table 8.6. Insulin Levels in Diabetic Rats.

Type of Treatment	Insulin Levels (mIU/L)				
	Day	Vehicle	Piperine	Sitagliptin	Piperine + Sitagliptin
	0	11.92 ± 0.76	11.71 ± 0.42	11.88 ± 0.40	11.84 ± 0.45 ns
SDT	**1**	11.54 ± 0.79	13.50 ± 0.19	15.41 ± 0.14	16.34 ± 0.11 **
MDT	**3**	10.79 ± 0.49	14.86 ± 0.37	16.36 ± 0.17	17.28 ± 0.36 **
	7	10.02 ± 0.38	15.57 ± 0.23	18.13 ± 0.66	19.09 ± 0.40 **
	14	8.69 ± 0.50	16.38 ± 0.26	19.49 ± 0.26	20.34 ± 0.24 **
	21	7.52 ± 0.71	17.39 ± 0.20	20.55 ± 0.20	21.49 ± 0.19 **

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at **p< 0.01 when compared with sitagliptin alone



ns- non significant, significant at **p< 0.01 when compared with sitagliptin alone

Figure 8.4. Insulin levels in Diabetic Rats.

8.2. PD Interaction Study of Sitagliptin with Piperine in Rabbits

8.2.1. Sitagliptin and Piperine Interactions in Normal Rabbits: A Study of Blood Glucose Levels

Table 8.7. Blood Glucose Levels in Normal Rabbits.

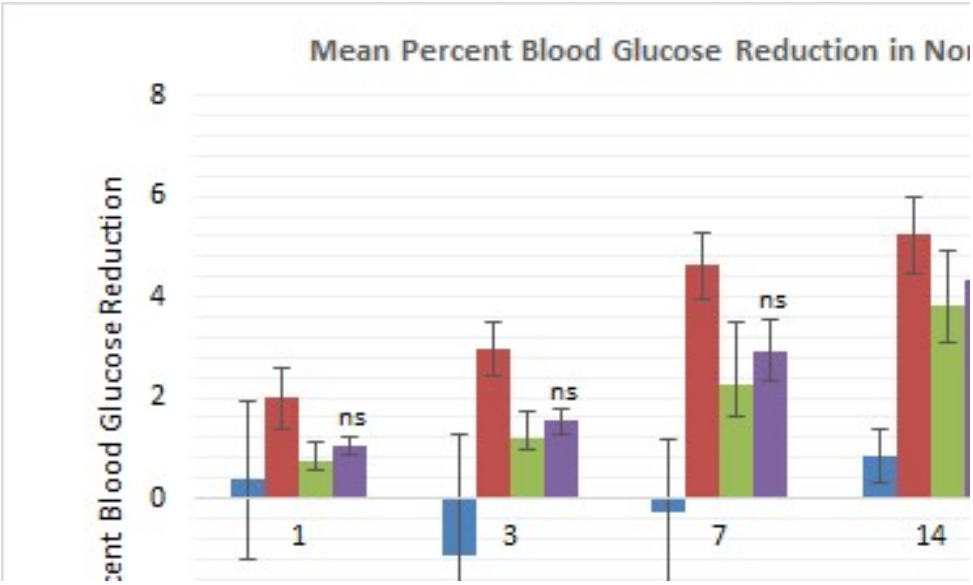
Type of Treatment	Blood Glucose Levels (mg/dl)				
	Day	Vehicle	Piperine	Sitagliptin	Piperine + Sitagliptin
	0	106.62 ± 1.44	107.92 ± 3.21	108.75 ± 2.11	108.63 ± 0.95 ns
SDT	**1**	106.22 ± 1.48	105.76 ± 3.48	107.94 ± 2.39	107.48 ± 0.83 ns
MDT	**3**	107.79 ± 1.67	104.70 ± 3.20	107.44 ± 2.37	106.97 ± 0.89 ns
	7	106.93 ± 0.67	102.92 ± 2.43	106.31 ± 3.26	105.43 ± 0.99 ns
	14	105.71 ± 0.91	102.26 ± 2.31	104.58 ± 2.98	103.90 ± 0.61 ns
	21	105.38 ± 0.84	101.52 ± 2.39	103.45 ± 2.62	102.02 ± 0.79 ns

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non-significant when compared with sitagliptin alone

Table 8.8. Percent Blood Glucose Reduction in Normal Rabbits.

Type of Treatment	Percent Blood Glucose Reductions (Mean ± SD)				
	Day	Vehicle	Piperine	Sitagliptin	Piperine + Sitagliptin
SDT	**1**	0.36 ± 1.58	2.00 ± 0.61	0.75 ± 0.36	1.06 ± 0.18 ns
MDT	**3**	-1.12 ± 2.39	2.98 ± 0.54	1.21 ± 0.52	1.53 ± 0.25 ns
	7	-0.30 ± 1.45	4.62 ± 0.64	2.26 ± 1.26	2.94 ± 0.63 ns
	14	0.84 ± 0.55	5.23 ± 0.76	3.85 ± 1.05	4.35 ± 0.75 ns
	21	1.15 ± 1.75	5.91 ± 0.67	4.89 ± 0.71	6.08 ± 0.73 ns

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non-significant when compared with sitagliptin alone



ns- non-significant when compared with sitagliptin alone

Figure 8.5. Mean Percent Blood Glucose Reduction in Normal Rabbits.

8.2.2. Sitagliptin and Piperine Interactions in Diabetic Rabbits: A Study of Blood Glucose Levels

Table 8.9. Blood Glucose Levels in Diabetic Rabbits.

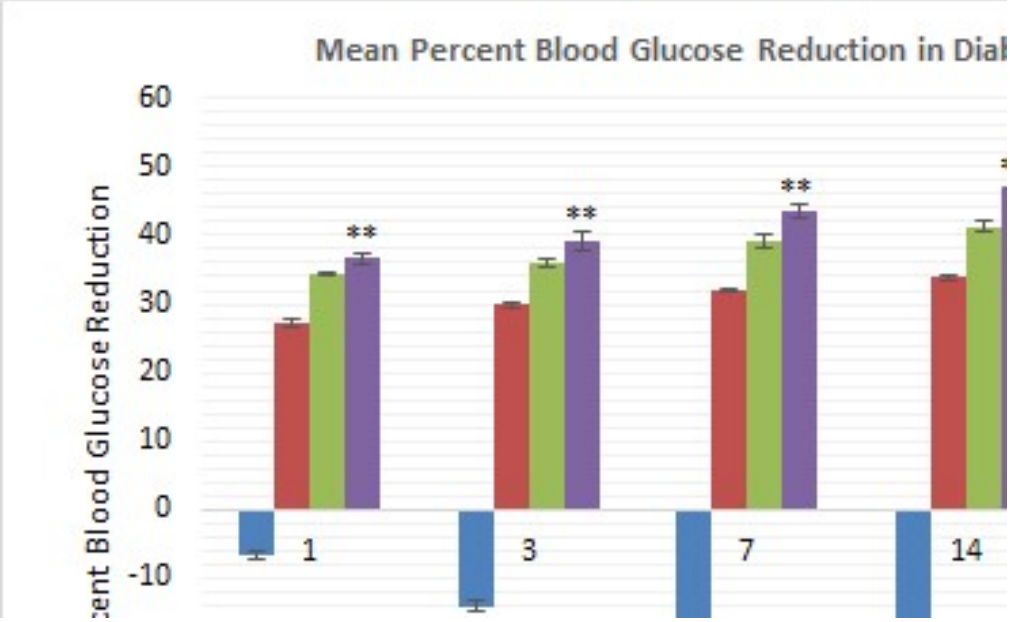
Type of Treatment	Blood Glucose Levels (mg/dl)				
	Day	Vehicle	Piperine	Sitagliptin	Piperine + Sitagliptin
	0	284.11 ± 1.94	284.85 ± 1.32	281.77 ± 1.38	283.16 ± 1.77 ns
SDT	**1**	303.00 ± 3.55	207.57 ± 1.47	185.17 ± 0.88	179.43 ± 2.16 *
MDT	**3**	324.12 ± 2.81	199.78 ± 1.11	180.24 ± 0.88	171.84 ± 3.39 **
	7	358.60 ± 5.71	193.6 ± 1.26	171.33 ± 2.15	159.87 ± 2.16 **
	14	371.03 ± 3.49	188.53 ± 0.93	156.88 ± 1.43	149.92 ± 1.26 **
	21	392.06 ± 4.68	182.28 ± 0.65	148.59 ± 2.35	141.01 ± 1.51 **

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at *p< 0.05, **p< 0.01 when compared with sitagliptin alone

Table 8.10. Percent Blood Glucose Reduction in Diabetic Rabbits

Type of Treatment	Percent Blood Glucose Reductions (Mean ± SD)				
	Day	Vehicle	Piperine	Sitagliptin	Piperine + Sitagliptin
SDT	**1**	-6.65 ± 0.63	27.13 ± 0.57	34.28 ± 0.15	36.63 ± 0.94 **
MDT	**3**	-14.08 ± 0.82	29.87 ± 0.43	36.03 ± 0.57	39.31 ± 1.43 **
	7	-26.23 ± 2.45	32.03 ± 0.31	39.19 ± 0.85	43.54 ± 0.83 **
	14	-30.59 ± 1.05	33.81 ± 0.41	41.32 ± 0.75	47.05 ± 0.31 **
	21	-37.99 ± 0.94	36.01 ± 0.30	47.26 ± 1.07	50.20 ± 0.39 ***

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at *p< 0.05 when compared with sitagliptin alone



ns- non significant, significant at **p< 0.01 when compared with sitagliptin alone

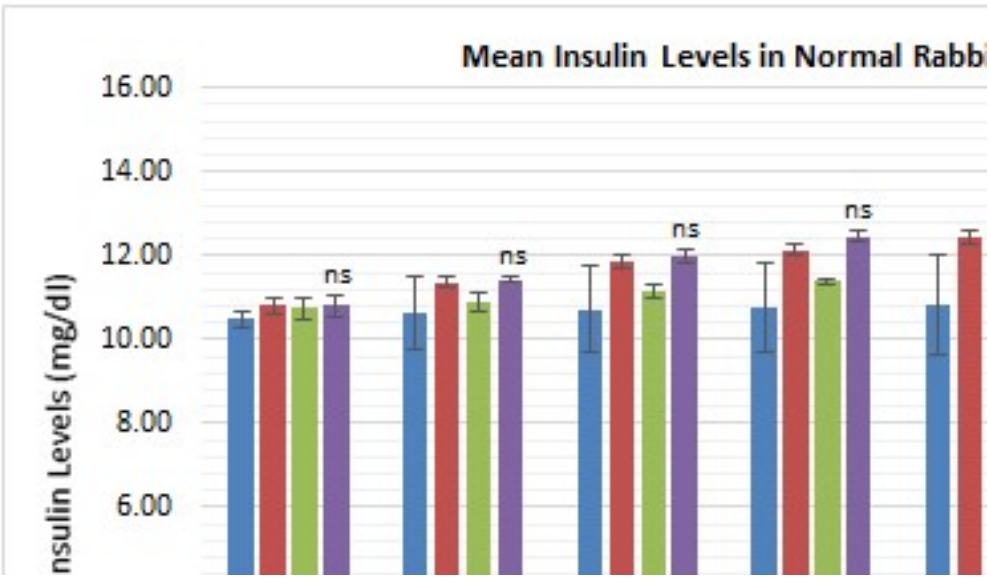
Figure 8.6. Mean Percent Blood Glucose Reduction in Diabetic Rabbits.

8.2.3. Sitagliptin and Piperine Interactions in Normal Rabbits: A Study of Insulin Levels

Table 8.11. Insulin Levels in Normal Rabbits.

Type of Treatment	Insulin Levels (mIU/L)				
	Day	Vehicle	Piperine	Sitagliptin	Piperine + Sitagliptin
SDT	0	10.48 ± 0.21	10.80 ± 0.19	10.74 ± 0.24	10.74 ± 0.24 ns
	1	10.63 ± 0.87	11.37 ± 0.12	10.88 ± 0.22	11.44 ± 0.08 ns
MDT	3	10.72 ± 1.03	11.88 ± 0.17	11.16 ± 0.15	11.96 ± 0.16 ns
	7	10.76 ± 1.04	12.15 ± 0.11	11.38 ± 0.05	12.47 ± 0.14 ns
	14	10.83 ± 1.22	12.45 ± 0.14	11.52 ± 0.11	12.77 ± 0.19 ns
	21	11.20 ± 1.42	12.94 ± 0.08	11.82 ± 0.11	13.15 ± 0.24 ns

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non-significant when compared with sitagliptin alone



ns- non-significant when compared with sitagliptin alone

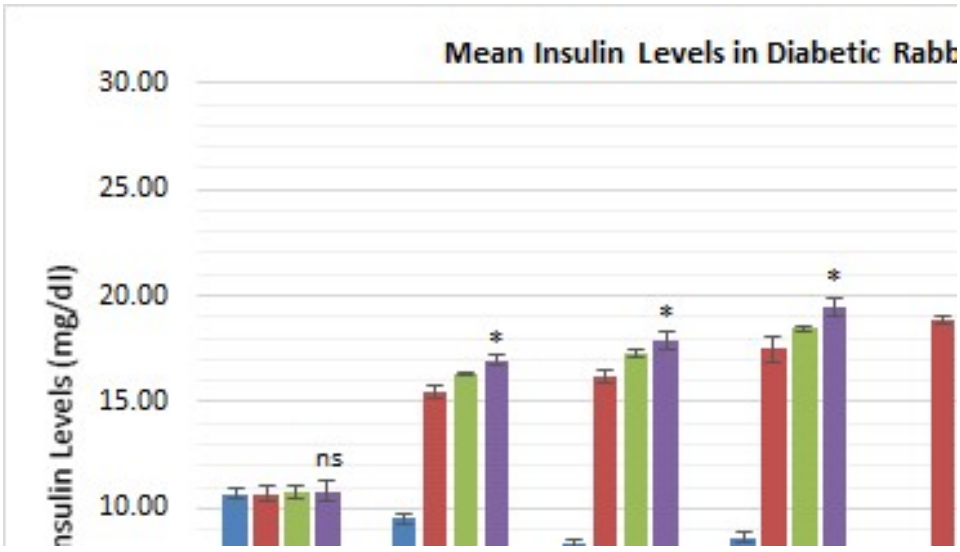
Figure 8.7. Insulin Levels in Normal Rabbits.

8.2.4. Sitagliptin and Piperine Interactions in Diabetic Rabbits: A Study of Insulin Levels

Table 8.12. Insulin Levels in Diabetic Rabbits.

Type of Treatment	Insulin Levels (mIU/L)				
	Day	Vehicle	Piperine	Sitagliptin	Piperine + Sitagliptin
	0	10.67 ± 0.26	10.68 ± 0.37	10.74 ± 0.34	10.79 ± 0.46 ns
SDT	**1**	9.49 ± 0.29	15.51 ± 0.32	16.32 ± 0.10	16.97 ± 0.21 *
MDT	**3**	8.38 ± 0.20	16.24 ± 0.29	17.26 ± 0.18	17.90 ± 0.41 *
	7	8.64 ± 0.22	17.49 ± 0.61	18.44 ± 0.13	19.46 ± 0.41 *
	14	7.19 ± 0.10	18.82 ± 0.20	21.15 ± 0.58	21.82 ± 0.14 *
	21	7.30 ± 0.64	19.98 ± 0.54	22.65 ± 0.08	23.76 ± 0.11 *

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant, significant at *p< 0.05 when compared with sitagliptin alone



ns- non significant, significant at *p< 0.05 when compared with sitagliptin alone

Figure 8.8. Insulin Levels in Diabetic Rabbits.

8.3. Discussion: PD interactions of Sitagliptin with Piperine

The phrase "PD interactions" implies interactions in which two drugs' directly influence each other [251]. Synergistic, additive, or antagonistic HDIs are frequently used to classify them. The mechanism of interaction with a complicated pathophysiological system might happen at the same target or via different paths [252].

This study intended to assess single and multiple-dose of sitagliptin to understand the impact of piperine in both rats and rabbits under normal and diabetic conditions.

Piperine alone has no hypoglycemic action in normal rats and normal rabbits, resulting in a very slight drop in glucose levels (tables 8.1 & 8.7), a very slight rise in percent blood glucose reduction (tables 8.2 & 8.8), and insulin (tables 8.5 & 8.11) on day 21 compared to day 1. However, when diabetic rats and diabetic rabbits are given piperine alone, they demonstrate anti hyperglycemic action by showing decreased blood glucose levels (tables 8.3 & 8.9), increased percent blood glucose reduction (tables 8.4 & 8.10), and higher insulin levels (tables 8.6 & 8.12) on day 21 compared to day 1. Sitagliptin alone shows antihyperglycemic action in diabetic animals (rats and rabbits) (tables 8.3 & 8.10) on days 1 and 21 but no hypoglycemic activity in normal rats and normal rabbits (tables 8.1 & 8.7). Sitagliptin alone therapy in diabetic animals (rats and rabbits) resulted in higher percent blood glucose reduction (tables 8.3 & 8.9) and higher insulin levels (tables 8.6 & 8.12) on days 1 and 21, but no changes in percent glucose reduction (tables 8.2 & 8.8) and insulin levels (tables 8.5 & 8.11) in normal animals (rats and rabbits) on days 1 and 21. However, when compared to a single-dose study in diabetic animals (rats and rabbits), the results show that a multiple-dose study of piperine alone and sitagliptin alone treatment is more effective in lowering blood glucose levels (tables 8.1, 8.3, 8.4 & 8.9), increasing percent blood glucose reduction (tables 8.2, 8.4, 8.8 & 8.10), and increasing insulin levels (tables 8.5, 8.6, 8.11 & 8.12) than a single-dose study in normal animals (rats and rabbits).

Co-administration of sitagliptin with piperine was shown to maintain anti-hyperglycemic effects in diabetic animals (rats and rabbits) from day 1 to day 21 (tables 8.3 & 8.9) but no hypoglycemic effects in normal animals (rats and rabbits) (tables 8.1 & 8.7) in this PD investigation.

The percent glucose reduction levels of single-dose treatment for combined therapy of sitagliptin and piperine (on day 1) in normal rats and normal rabbits were observed to be 1.18 ± 0.85 and 1.06 ± 0.18, respectively, as compared with sitagliptin alone therapy was observed to be 1.03 ± 0.96 and 0.75 ± 0.36 respectively (table 8.2 & 8.7).

The percent glucose reduction levels of MDT for combined therapy of sitagliptin and piperine (on day 21) in normal rats and normal rabbits were observed to be 5.6 ± 3.49 and 6.08 ± 0.73, respectively, as compared with sitagliptin alone therapy was observed to be 5.23 ± 3.99 and 5.84 ± 1.46 respectively (table 8.2 & 8.7).

The percent glucose reduction levels of SDT for combined therapy of sitagliptin and piperine (on day 1) in diabetic rats and diabetic rabbits were observed to be 45.82 ± 1.87 and 36.63 ± 0.94, respectively, as compared with sitagliptin alone therapy was observed to be 40.9 ± 1.01 and 34.28 ± 0.15 respectively (tables 8.4 & 8.10).

The percent glucose reduction levels of multiple-dose treatment for combined therapy of sitagliptin and piperine (on day 21) in diabetic rats and diabetic rabbits were observed to be 57.50 ± 2.14 and 50.20 ± 0.39, respectively, as compared with sitagliptin alone therapy was observed to be 52.18 ± 0.53 and 47.26 ± 1.07 respectively (tables 8.4 & 8.10).

In normal animals (rats and rabbits), sitagliptin along with piperine treatment had no significant outcome on blood glucose levels (tables 8.1 & 8.7), percent blood glucose reduction (table 8.2 & 8.5), and insulin levels (tables 8.5 & 8.11) as compared to sitagliptin alone therapy. In diabetic animals (rats and rabbits), sitagliptin along with piperine treatment resulted in significantly ($p<0.05$) lower blood glucose levels (tables 8.3 & 8.9), significantly ($p<0.01$) higher percent blood glucose reduction (tables 8.4 & 8.10) and significantly ($p<0.001$) enhanced insulin levels

(tables 8.6 & 8.12) when compared to sitagliptin alone therapy for both single and multiple-dose treatment. However, in diabetic animals (rats and rabbits), multiple-dose treatment was more beneficial than single dosage therapy.

Sitagliptin works by prolonging the action of proteins that enhance insulin release when blood sugar levels rise, such as after food. Sitagliptin is a selective inhibitor of the enzyme DPP-4, which metabolizes the naturally arising incretin hormones GIP and GLP-1 production. GLP-1 stimulates insulin secretion (acting as an incretin hormone) while inhibiting glucagon release, helping to minimize postprandial glucose rise. GLP-1 has various additional effects, including slowing gastric emptying, lowering glucagon levels, and perhaps influencing hunger in the central nervous system. Sitagliptin is readily absorbed after oral administration, and it is processed by CYP P450 3A4 and 2C8 substrates and transported via P-gp [176, 177].

Piperine is a thermonutrient herb that may help nutrient absorption by increasing thermogenesis. The autonomous nervous system represents two major receptors in the GIT, the α- and β-adrenergic receptors, according to the concept of piperine-induced thermogenesis [196]. According to Majeed *et al*. patent on piperine [254], piperine exhibits β agonistic action on adrenergic receptors. β-receptors, which contain cyclic adenosine 3', 5' monophosphate, enhance piperine-induced thermogenesis (cAMP). The importance of cAMP as a "second messenger" in the body's hormonal and enzymatic activities is well understood. The need for new nutrients to maintain metabolic activities increases fast during thermogenesis [196]. The activation of β-receptors increases the proliferation of β-cells and their ability to produce insulin. Blood glucose levels decline due to the released insulin [255]. As a result, piperine's acute effects might be explained by a selective or relatively strong agonism at these receptors. Based on recent studies, piperine is a selective inhibitor of CYP P450 enzyme isoforms, i.e. CYP1A2, CYP1A1, CYP2D6, CYP3A4, and CYP2C8, as well as inhibiting P-gp, a key efflux pump [256]. Piperine has also been shown to affect the absorption of co-administered medicines in the intestine, resulting in drug interactions [257, 258].

When piperine was given along with sitagliptin, the results revealed an additive effect in diabetic animals (rats and rabbits) by showing significantly ($p<0.001$) increased percent blood glucose reduction (tables 8.4 & 8.10) and significantly ($p<0.05$, $p<0.01$) raised insulin levels (tables 8.7 & 8.12) when compared to sitagliptin alone treatment. There is no significant outcome on percent blood glucose reduction (tables 8.3 & 8.9) and insulin levels (tables 8.5 & 8.12) in normal animals (rats and rabbits) when treated with sitagliptin and piperine.

According to previous research, Sitagliptin has a high absorption rate and a short half-life [257, 258]. According to the literature, sitagliptin inhibits DPP-4 from metabolizing GLP-1, increasing insulin synthesis, whereas piperine activates the β-receptors of β-cells which cause more insulin release. The study results demonstrated that sitagliptin in the presence of piperine had an additive impact, significantly ($p<0.05$, $p<0.01$) releasing more insulin (tables 8.6 & 8.12) and significantly ($p<0.05$, $p<0.01$) lowering glucose levels (tables 8.3 & 8.9) in diabetic rats and diabetic rabbits. In single and multiple-dose studies, sitagliptin with piperine had no significant hypoglycemia effect in normal rats and normal rabbits (table 8.1, 8.5, 8.7 & 8.11).

Piperine may reduce blood glucose levels after co-administration of metformin owing to improved gastrointestinal absorption, which is likely related to increased micelle production, gastrointestinal flow, and higher permeability, which aids in drug uptake [261]. Piperine also reduced nateglinide & glimepiride blood glucose levels, likely related to metabolic inhibition of the CYP 3A4 and 2C9 enzymes [260]. Following a review of the literature, the study reveals that piperine enhanced gastrointestinal absorption when treated along with sitagliptin and inhibited the CYP 3A4 and 2C8 isoform enzymes, as well as the P-gp transporter of sitagliptin, through a PD mechanism. This study demonstrates that piperine impacted sitagliptin absorption and metabolism in the intestine, resulting in a piperine-sitagliptin drug interaction.

O. Conner *et al.* discussed that administration of dietary supplements in capsule form (which includes one of the ingredients, i.e., piperine) in subjects showed elevated levels of GLP-1, which can enhance insulin release [266]. Until now, there is no evidence that piperine alone has elevated the GLP-1 levels. If piperine shows its

influence on an elevation of GLP-1 levels in future studies, then this study can assume that piperine with sitagliptin may synergistically influence GLP-1 to enhance insulin levels.

A research study on volunteers treated with curcumin and piperine, and the results showed with 0.1 percent reduction in HbA1C after four months [267].

Conclusion

This study's results confirmed the PD interaction of sitagliptin and piperine in diabetic animals (rats and rabbits) but not in normal animals (rats and rabbits).

The study results showed a significant anti hyperglycemic action in diabetic animals (rats and rabbits) and no hypoglycemic impact in normal animals (rats and rabbits) when sitagliptin was co-administered with piperine.

Because the interaction was observed in two different diabetic species, it is probable to happen in humans as well, necessitating dose adjustments. Therefore, care should be taken while recommending this combination for therapeutic benefit.

8.4. PK Interaction Study of Sitagliptin with Piperine in Rabbits

8.4.1. PK Interaction Study of Sitagliptin with Piperine in Normal Rabbits

Table 8.13. Mean Plasma Concentration of Sitagliptin in Normal Rabbits after treatment with Sitagliptin in the absence and presence of Piperine on each Day 1 and Day 21

Time/Hr	Day 1 (SDT)		Day 21 (MDT)	
	Sitagliptin	**Piperine + Sitagliptin**	**Sitagliptin**	**Piperine + Sitagliptin**
0	0 ± 0.00	0 ± 0	0 ± 0	0 ± 0
1	0.55 ± 0.16	1.05 ± 0.33	0.62 ± 0.07	0.85 ± 0.11
2	2.27 ± 0.62	3.44 ± 0.64	2.65 ± 0.41	3.58 ± 0.26
3	3.35 ± 0.51	4.46 ± 0.37	3.67 ± 0.71	4.88 ± 0.311
4	1.83 ± 0.57	2.96 ± 0.58	1.99 ± 0.43	2.78 ± 0.11
8	0.6 ± 0.32	1.3 ± 0.30	0.79 ± 0.18	1.31 ± 0.28
16	0.35 ± 0.06	0.7 ± 0.19	0.30 ± 0.08	0.48 ± 0.09
24	0.15 ± 0.04	0.29 ± 0.08	0.15 ± 0.02	0.25 ± 0.04

SDT- Single-dose treatment, MDT-Multiple-dose treatment

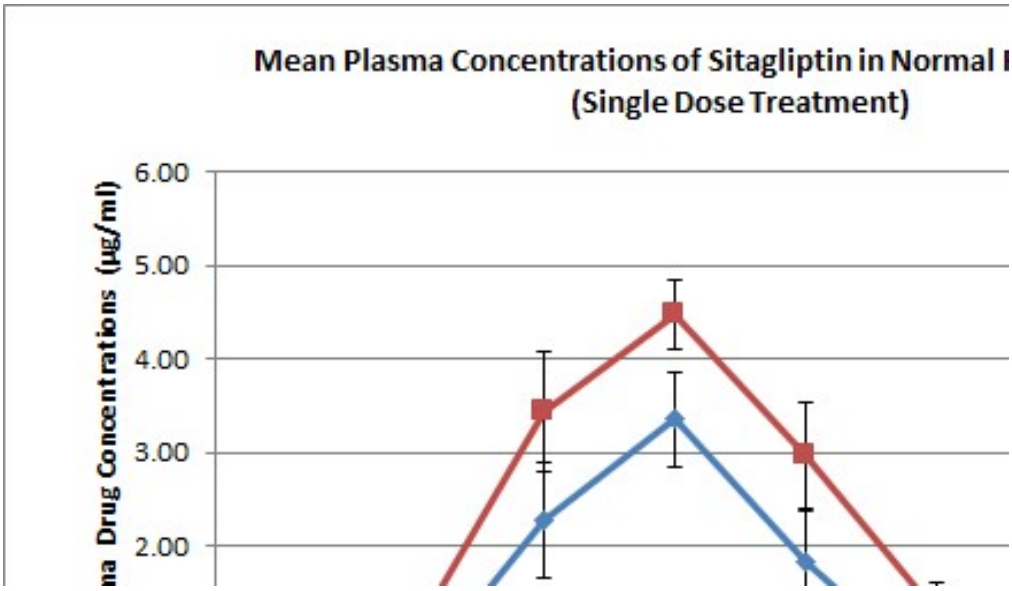


Figure 8.9. Mean Plasma Concentrations of Sitagliptin in Normal Rabbits after treatment with Sitagliptin in the absence and presence of Piperine on Day 1

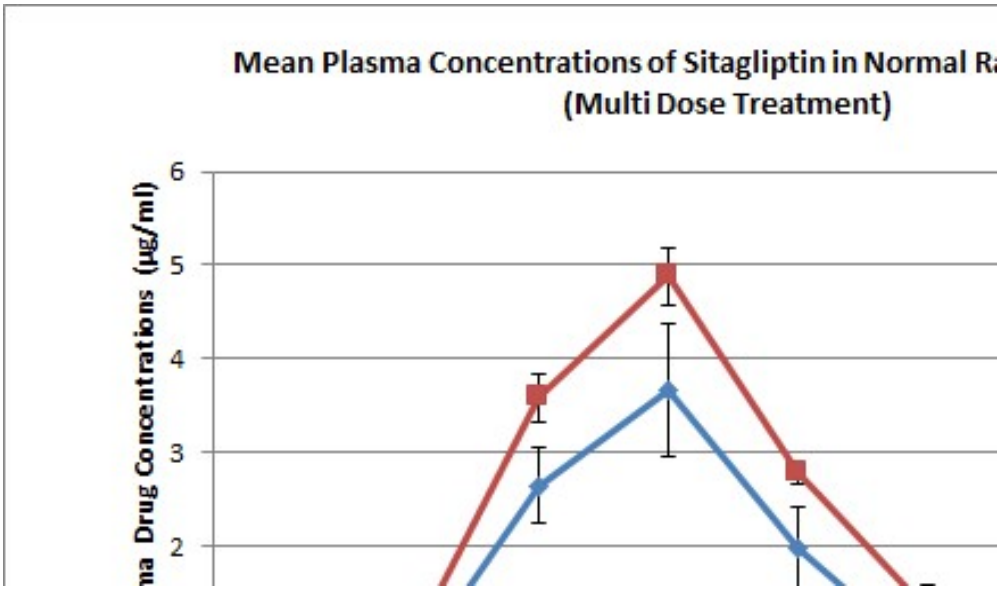


Figure 8.10: Mean Plasma Concentration of Sitagliptin in Normal Rabbits after treatment with Sitagliptin in the absence and presence of Piperine on Day 21

Table 8.14. Mean PK Parameters of Sitagliptin in Normal Rabbits after treatment with Sitagliptin in the absence and presence of piperine on each Day 1 and Day 21

PK Parameters	Day 1 (SDT)		Day 21 (SDT)	
	Sitagliptin	Piperine + Sitagliptin	Sitagliptin	Piperine + Sitagliptin
C_{max} (ng/ml)	3.35 ± 0.51	4.46 ± 0.37 *	3.67 ± 0.71	4.88 ± 0.31*
T_{max} (h)	3 ± 0.00	3 ± 0.00 ns	3 ± 0.00	3 ± 0.00 ns
AUC_{0-t} (ng/ml*h)	17.73 ± 1.10	30.95 ± 5.45 **	19.69 ± 2.33	28.97 ± 2.00 ***
$AUC_{0-\infty}$ (ng/ml*h)	19.33 ± 1.80	34.03 ± 6.31 **	21.12 ± 2.22	31.47 ± 1.81 ***
$AUMC_{0-t}$ (ng/ml*h^2)	121.24 ± 17.02	231.15 ± 51.82 **	128.08 ± 15.86	198.94 ± 20.57 **
$AUMC_{0-\infty}$ (ng/ml*h^2)	178.37 ± 51.50	338.14 ± 89.67 *	176.09 ± 10.35	284.67 ± 36.11 **
MRT_{0-t} (h)	6.81 ± 0.56	7.42 ± 0.47 ns	6.52 ± 0.49	6.86 ± 0.35 ns
$MRT_{0-\infty}$ (h)	9.11 ± 1.81	9.84 ± 1.08 ns	8.37 ± 0.51	9.04 ± 1.02 ns
K_e (1/h)	0.11 ± 0.03	0.1 ± 0.02 ns	0.11 ± 0.02	0.11 ± 0.02 ns
$T_{1/2}$ (h)	7.1 ± 2.47	7.13 ± 1.23 ns	6.54 ± 1.35	6.78 ± 0.51 ns
CL (mg/kg)/(ng/ml)/h	0.36 ± 0.03	0.21 ± 0.04 **	0.33 ± 0.03	0.22 ± 0.01 ***
Vd (L)	3.66 ± 0.99	2.17 ± 0.56 *	3.16 ± 0.75	2.18 ± 0.53 ns

SDT- Single-dose treatment, MDT-Multiple-dose treatment

ns- non significant, significant at *p< 0.05, **p< 0.01, ***p< 0.001 when compared with sitagliptin alone

Table 8.15. Mean PK Parameters of Sitagliptin with piperine in Normal Rabbits on Day 1 and Day 21

PK Parameters	Day 1 (SDT)	Day 21 (SDT)
	Piperine + Sitagliptin	Piperine + Sitagliptin
C_{max} (ng/ml)	4.46 ± 0.37	4.88 ± 0.31 ns
T_{max} (h)	3 ± 0.00 ns	3 ± 0.00 ns
AUC_{0-t} (ng/ml*h)	30.95 ± 5.45	28.97 ± 2.00 ns
$AUC_{0-\infty}$ (ng/ml*h)	34.03 ± 6.31	31.47 ± 1.81 ns
$AUMC_{0-t}$ (ng/ml*h^2)	231.15 ± 51.82	198.94 ± 20.57 ns
$AUMC_{0-\infty}$ (ng/ml*h^2)	338.14 ± 89.67	284.67 ± 36.11 ns
MRT_{0-t} (h)	7.42 ± 0.47	6.86 ± 0.35 ns
$MRT_{0-\infty}$ (h)	9.84 ± 1.08	9.04 ± 1.02 ns
K_e (1/h)	0.1 ± 0.02	0.11 ± 0.02 ns
$T_{1/2}$ (h)	7.13 ± 1.23	6.78 ± 0.51 ns
CL (mg/kg)/(ng/ml)/h	0.21 ± 0.04	0.22 ± 0.01 ns
Vd (L)	2.17 ± 0.56	2.18 ± 0.53 ns

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant when compared with day 1

8.4.2 PK Interactions study of Sitagliptin with Piperine in Diabetic Rabbits

Table 8.16. Mean Plasma Drug Concentrations of Sitagliptin in Diabetic Rabbits after treatment with Sitagliptin in the absence and presence of Piperine on each Day 1 and Day 21

Time/Hr	Day 1 (SDT)		Day 21 (MDT)	
	Sitagliptin	Piperine + Sitagliptin	Sitagliptin	Piperine + Sitagliptin
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1	0.69 ± 0.06	0.94 ± 0.19	0.7 ± 0.05	0.92 ± 0.16
2	2.48 ± 0.47	2.77 ± 0.48	2.59 ± 0.39	3.32 ± 0.28
3	3.5 ± 0.74	5.11 ± 0.84	3.54 ± 0.75	5.3 ± 0.76
4	2.01 ± 0.48	2.95 ± 0.17	2.17 ± 0.45	3.42 ± 0.68
8	0.57 ± 0.20	0.9 ± 0.13	0.54 ± 0.19	1.09 ± 0.30
16	0.26 ± 0.08	0.48 ± 0.13	0.29 ± 0.06	0.55 ± 0.11
24	0.17 ± 0.02	0.31 ± 0.09	0.18 ± 0.03	0.28 ± 0.03

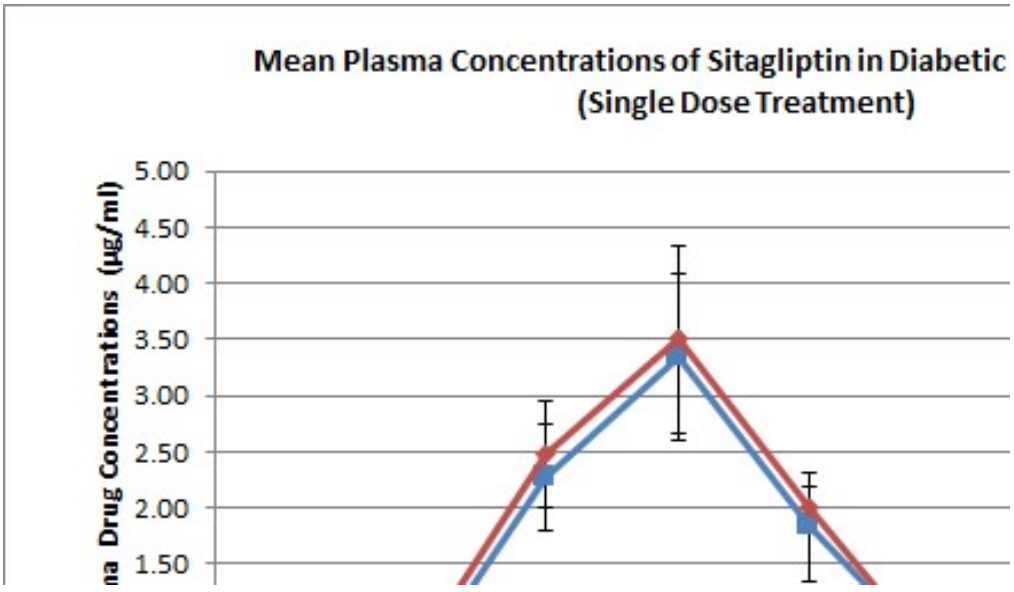


Figure 8.11. Mean Plasma Drug Concentrations of Sitagliptin in Diabetic Rabbits after treatment with Sitagliptin in the absence and presence of Piperine on Day 1

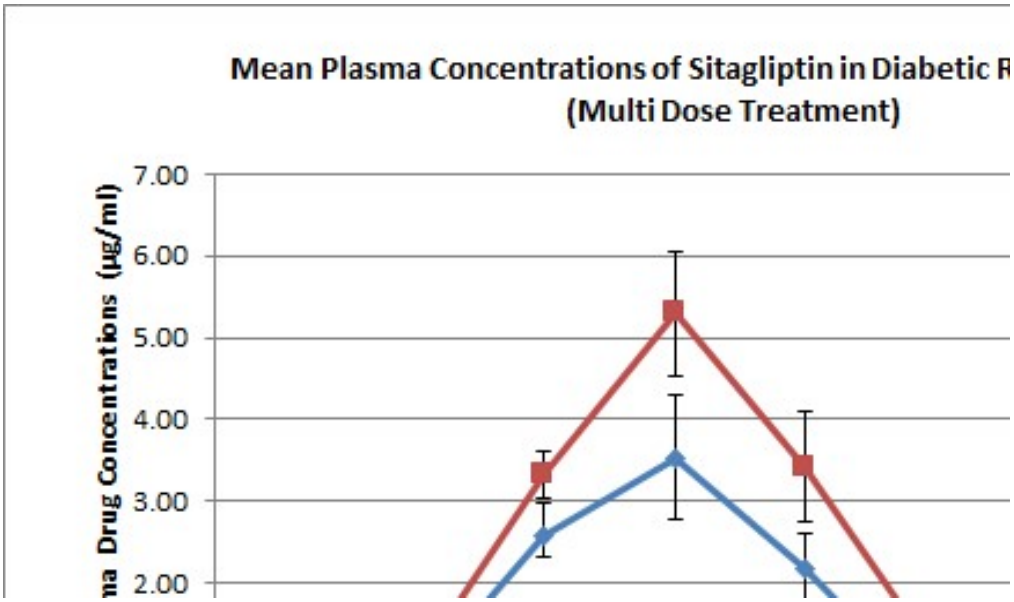


Figure 8.12. Mean Plasma Drug Concentrations of Sitagliptin in Diabetic Rabbits after treatment with Sitagliptin in the absence and presence of Piperine on Day 21

Table 8.17. Mean PK Parameters of Sitagliptin in Diabetic Rabbits after treatment with Sitagliptin alone and Sitagliptin in presence of Piperine on each Day 1 and Day 21

PK Parameters	Day 1 (SDT)		Day 21 (MDT)	
	Sitagliptin	Piperine + Sitagliptin	Sitagliptin	Piperine + Sitagliptin
C_{max} (ng/ml)	3.5 ± 0.74	5.11 ± 0.84 *	3.54 ± 0.75	5.37 ± 0.76 *
T_{max} (h)	3 ± 0.00	3 ± 0.00 ns	3 ± 0.00	3 ± 0.00 ns
AUC_{0-t} (ng/ml*h)	17.82 ± 2.70	26.63 ± 3.70 **	18.55 ± 1.07	30.18 ± 5.06 **
$AUC_{0-\infty}$ (ng/ml*h)	20.09 ± 3.05	31.49 ± 5.30 **	21.51 ± 1.29	33.77 ± 4.48 **
$AUMC_{0-t}$ (ng/ml*h^2)	112.48 ± 19.66	184.9 ± 33.56 ns	118.85 ± 12.73	207.15 ± 36.94**
$AUMC_{0-\infty}$ (ng/ml*h^2)	202.5 ± 62.24	382.05 ± 135.91 ns	240.27 ± 50.05	340.84 ± 42.27 *
MRT_{0-t} (h)	6.32 ± 0.64	6.92 ± 0.43 ns	6.42 ± 0.74	6.86 ± 0.19 ns
$MRT_{0-\infty}$ (h)	10.02 ± 2.40	11.91 ± 2.77 ns	11.14 ± 1.96	10.18 ± 1.56 ns
K_e (1/h)	0.09 ± 0.05	0.07 ± 0.02 ns	0.07 ± 0.02	0.08 ± 0.02 ns
$T_{1/2}$ (h)	9.3 ± 4.46	10.54 ± 2.53 ns	11.24 ± 2.98	8.7 ± 1.97 ns
CL (mg/kg)/(ng/ml)/h	0.35 ± 0.06	0.23 ± 0.04 **	0.33 ± 0.02	0.21 ± 0.02 ***
Vd (L)	4.68 ± 2.08	3.39 ± 0.67 ns	5.26 ± 1.29	2.67 ± 0.81 *

SDT- Single-dose treatment, MDT-Multiple-dose treatment

ns- non significant, significant at *p< 0.05, **p< 0.01, ***p< 0.001 when compared with sitagliptin alone

Table 8.18. Mean PK Parameters of Sitagliptin with piperine in Diabetic Rabbits Day 1 and Day 21

PK Parameters	Day 1 (SDT)	Day 21 (MDT)
	Piperine + Sitagliptin	**Piperine + Sitagliptin**
C_{max} (ng/ml)	5.11 ± 0.84	5.37 ± 0.76 ns
T_{max} (h)	3 ± 0.00	3 ± 0.00 ns
AUC_{0-t} (ng/ml*h)	26.63 ± 3.70	30.18 ± 5.06 ns
$AUC_{0-\infty}$ (ng/ml*h)	31.49 ± 5.30	33.77 ± 4.48 ns
$AUMC_{0-t}$ (ng/ml*h^2)	184.9 ± 33.56	207.15 ± 36.94 ns
$AUMC_{0-\infty}$ (ng/ml*h^2)	382.05 ± 135.91	340.84 ± 42.27 ns
MRT_{0-t} (h)	6.92 ± 0.43	6.86 ± 0.19 ns
$MRT_{0-\infty}$ (h)	11.91 ± 2.77 ns	10.18 ± 1.56 ns
K_e (1/h)	0.07 ± 0.02 ns	0.08 ± 0.02 ns
$T_{1/2}$ (h)	10.54 ± 2.53 ns	8.7 ± 1.97 ns
CL (mg/kg)/(ng/ml)/h	0.23 ± 0.04 **	0.21 ± 0.02 ns
Vd (L)	3.39 ± 0.67 ns	2.67 ± 0.81 ns

SDT- Single-dose treatment, MDT-Multiple-dose treatment
ns- non significant when compared with day 1

8.5. Discussion: PK interaction of Sitagliptin with Piperine

The study findings may be attributable to piperine's bio-enhancing properties when combined with sitagliptin (tables 8.15 & 8.18). Bioenhancers are chemical entities that, when combined with drugs, increase their bioavailability without a synergistic impact on the drug [268]. Interacting substrate specificity in physiologic systems' biotransformational pathways is the primary cause of HDI [192], which can be a crucial step in optimizing the use of piperine with sitagliptin, particularly in the management of chronic illnesses, such as DM.

This study compared single and multiple doses of sitagliptin to explore the impact of piperine in normal and diabetic rabbits. Piperine has been proved to increase the bioavailability of a wide variety of drugs. Sitagliptin bioavailability alterations in the presence of piperine were studied in normal and diabetic rabbits.

In normal rabbits

The plasma concentration-time profile of sitagliptin (figure 8.9 & 8.10) after oral administration in the absence and presence of piperine was found to be a significant difference in normal rabbits on day 1 and day 21 and is presented in table 8.14. The PK parameters of sitagliptin in the absence and presence of piperine in normal rabbits on day 1 and day 21 are summarized in table 8.15.

On day 1, the C_{max} of sitagliptin, i.e., 3.35 ± 0.51 ng/ml, was obtained in the absence of piperine, and 4.46 ± 0.37 ng/ml was obtained in the presence of piperine in normal rabbits (table 8.15). This study represents a significant ($p<0.05$) increase of 33.13 % in peak plasma levels of sitagliptin. Other PK parameters by comparing the bioavailability of sitagliptin in the absence and presence of piperine are given in table 8.15 of day 1. An increased C_{max} and reduced clearance contributed to an apparent increase in AUC. $T_{1/2}$ remains unchanged with no significant difference. In a single-dose study, the results suggest that piperine significantly enhances sitagliptin bioavailability.

On day 21, the C_{max} of sitagliptin, i.e., 3.67 ± 0.71 ng/ml, was obtained in the absence of piperine, and 4.88 ± 0.31 ng/ml was obtained in the presence of piperine in

normal rabbits (table 8.14). This study represents a significant ($p<0.05$) rise of 32.97 % in peak plasma levels of sitagliptin. Other PK parameters by comparing the bioavailability of sitagliptin in the absence and presence of piperine are given in table 8.15 of day 21. An increased C_{max} and reduced clearance contributed to an apparent increase in AUC. $T_{1/2}$ remains unchanged with no significant difference. The above findings show that piperine significantly improves the bioavailability of sitagliptin in the multiple-dose study.

In diabetic rabbits

The plasma concentration-time profile of sitagliptin (figures 8.11 & 8.12) after per oral administration of sitagliptin in the absence and presence of piperine was found to be a significant difference in diabetic rabbits on day 1 and day 21 and are presented in table 8.17. The PK parameters of sitagliptin in the absence and presence of piperine in diabetic rabbits on day 1 and day 21 are summarized in table 8.18.

On day 1, the C_{max} of sitagliptin was 3.5 ± 0.74 ng/ml was obtained in the absence of piperine, and 5.11 ± 0.84 ng/ml was obtained in the presence of piperine in diabetic rabbits (table 8.18). This study represents a significant ($p<0.05$) increase of 46.00 % in peak plasma levels of sitagliptin. Other PK parameters by comparing the bioavailability of sitagliptin in the absence and presence of piperine are given in table 8.18 of day 1. An increased C_{max} and reduced clearance contributed to an apparent increase in AUC. $T_{1/2}$ remains unchanged with no significant difference. The data above reveal that piperine significantly enhances sitagliptin bioavailability in a single-dose study.

On day 21, the C_{max} of piperine was 3.54 ± 0.75 ng/ml was obtained in the absence of piperine, and 5.37 ± 0.76 ng/ml was obtained in the presence of piperine in diabetic rabbits (table 8.17). This study represents a significant increase of 51.69 % in peak plasma levels of Sitagliptin. Other PK parameters by comparing the bioavailability of sitagliptin in the absence and presence of piperine are given in table 8.17 of day 21. An increased C_{max} and reduced clearance contributed to an apparent increase in AUC. $T_{1/2}$ remains unchanged with no significant difference. The

data above reveal that piperine significantly enhances sitagliptin bioavailability in a multiple-dose study.

The PK results for day 1 and day 21 in normal rabbits (table 8.14) and diabetic rabbits (table 8.18) clearly show that piperine did not affect sitagliptin's onset of action (T_{max}) but significantly ($p<0.01$, $p<0.001$, $p<0.0001$) augmented total plasma exposure (AUC and AUMC). Because of the increased gastrointestinal absorption, there might be an interaction. Increased absorption could result from (a) improved micelle formation, which leads to increased solubility, (b) improved gastrointestinal blood flow, (c) augmented permeability due to epithelial cell alteration, and (d) increased brush border membrane fluidity, which leads to increased microvilli length [268]. Piperine was shown to enhance bioavailability by 30 % to 200 % [269], and sitagliptin bioavailability is over 87 % [176], with no change in sitagliptin T_{max}. Sitagliptin has low protein binding to drugs, i.e., 38% [174], so there is a very low possibility of protein binding interaction. However, in normal rabbits, the Vd of sitagliptin was significantly ($p<0.05$) decreased on day 1 and non-significantly diminished on day 21 (table 8.15), whereas, in diabetic rabbits, the Vd of sitagliptin was significantly ($p<0.05$) decreased on day 21, and non-significantly decreased on day 1 (table 8.17), implying that the interaction may be fractionally involved in protein displacement. As a result, the rise in sitagliptin concentrations in the presence of piperine might be due to a change in metabolism or excretion.

Sitagliptin does not undergo immense metabolism, but the CYP system slightly converts sitagliptin to inactive metabolites via CYP3A4 and 2C8, and sitagliptin predominantly effluxes out by P-gp substrate transporter via urine [176, 177]. Piperine inhibits P-gp substrate primarily and CYP CYP3A4 partially in in-vivo, increasing the plasma/serum concentration levels of several drugs (e.g., azithromycin and carbamazepine) [220, 237]. Piperine also inhibits the P-gp of many drugs (e.g., linarin and fexofenadine) [216, 218], leading to increased plasma drug concentration levels. Piperine inhibits enzymes and P-gp, which has major therapeutic consequences since lower drug metabolism leads to higher drug concentration, which leads to improved sitagliptin bioavailability. Piperine's structure is responsible for its inhibitory effect on enzymes. The methylenedioxyphenyl (MDP)

ring, piperidine moiety and side chain all work together to block 7-methoxycoumarin-Odemethylase (MOCD) and aryl hydrocarbon hydroxylase (AHH) activities. Alteration of just one component in the piperine particle can cause differential inhibition of the two kinds of monooxygenase activity [268]. Human p-glycoprotein, a key efflux pump, is inhibited by piperine [192], but there is no clear data on how piperine will have a mechanistic process of P-gp inhibition.

Sitagliptin is primarily excreted unalterably, roughly around 79% in urine [176]. According to PK data, such as reduced Cl and no significant changes for Ke, MRT, or $T_{1/2}$, the current study indicates that piperine increases sitagliptin metabolism, which leads to delayed clearance and greater sitagliptin plasma concentrations.

Conclusion

1. In this study, MDT of sitagliptin had a greater effect on piperine activity (plasma concentration level and PKs) than Single-dose therapy in diabetic rabbits.
2. The study also concludes that piperine increased the bioavailability of sitagliptin.
3. Since the interaction was seen in rabbits, it is likely to occur in humans as well, leading to significantly enhanced sitagliptin activity and maybe require dosage adjustments. As a result, caution should be exercised when this combination is given for therapeutic benefit.

6. SUMMARY AND CONCLUSION

9.1 Summary

The World Health Organization pushed poorer nations to combine modern management with traditional herbal therapies in 1974 [238]. In India, sales of herbal products have continued to increase [4]. According to a survey conducted at an Indian tertiary care hospital, about 58 percent of doctors and 28% of patients use herbal therapy besides conventional medications [3]. These activities raise concerns about an augmented risk of adverse herbal-drug interactions (HDIs), defined as any change in the "victim" drug's PKs and/or PDs caused by a herbal product and can cause drug-related toxic effects or decreased effectiveness. HDIs can influence the PK and PD of co-administered conventional medicines, and most reported HDIs are PK-related [5]. Many ingredients in herbal products differ in composition across manufacturers and batches [273]. Herbal preparations may alter drug-metabolizing enzymes and transporters due to interactions with one or more of its constituents. Multiple components may interact in additive, synergistic, or antagonistic ways, resulting in a net effect [274].

DM is a disease in which the body does not produce sufficient insulin or does not react to it correctly, resulting in remarkably high blood sugar levels [15]. According to research, DM will impact 72.96 million adults in India. In urban cities, the prevalence varies from 10.9 percent to 14.2 percent. In contrast, in rural India, the prevalence ranged from 3.0 percent to 7.8 percent among the ages 20 and above, with a much greater incidence among those over 50 years old (INDIAB Study) [17]. According to the IDF, 285 billion individuals (6.4% adult population) were diagnosed with DM in 2010, and in 2030 expect this number to grow to 439 million globally [16]. A reduction in insulin production causes type-1 DM, whereas hyperglycemia marked type-2 DM in the presence of insulin resistance and relative insulin shortage [15, 18].

HDI is caused by overlapping substrate specificity in physiologic systems' bio-transformational pathways [275]. The ability of various chemical moieties to bind with receptor sites and alter the physiological state explains PD medication interactions. In contrast, alterations in absorption, intervention in distribution patterns,

and alterations in metabolic rate and excretory pathways contribute to PK drug interactions [83].

Because of its additive value, meat packers, canners, picklers, and bakers often use pepper. At the end of the process, it also changes the particular flavour of the food. In Indian medicine, black pepper is a necessary component [179]. Black and long pepper are used as household spices in many contemporary and traditional medical systems worldwide [180]. Piperine has a variety of pharmacological effects, according to published research, which might contribute to positive therapeutic outcomes [276]. Piperine has shown hepatoprotective, anti-inflammatory, anti-ulcer, antioxidant and antidiabetic properties [11, 192]. In this scenario, DM patients are more likely to include piperine in their normal diet, as piperine inhibits the CYP450 enzyme and P-gp, influencing β-cells that can release insulin. However, there is no report/evidence on the effect of piperine on the PDs and/or PKs of repaglinide and sitagliptin in terms of the combination's safety and effectiveness, which is critical in clinical practice to provide appropriate therapy.

HDI screening and prediction may be made using a variety of methodologies, including molecular biology, in silico methods, vitro models and high throughput screen assays, as part of the drug development process [192, 267, 277, 278]. These approaches provide a more convenient manner of obtaining HDIs data based only on drug-inducing and/or inhibitory efficacy with minimum resources. Still, it also linked them with several clinical constraints. Clinical HDIs are costly and require at least some accurate PD and PK in vivo data from pre-clinical research to evaluate potential HDIs and develop a practical clinical trial design. Furthermore, studying HDIs in various animal models by considering PDs and PKs is critical because they depict a variety of factors such as dose, potency, and drug concentration at the active site of receptors and drug-metabolizing enzymes, all of which are critical from a clinical standpoint. Thus, pharmacologists in the drug development process have sought to develop rapid, sensible, and animal models to evaluate HDIs, allowing clinicians to understand HDI mechanisms better and provide rational therapy to patients in terms of the safety and efficiency of drug blends, particularly in chronic illnesses such as DM.

Drug interactions are more common, and the causes of interactions are typically investigated using animal models. Though animal models can never substitute thorough human trials, they can help researchers better understand and assess drug interactions. It is possible to obtain a good prediction of drug PKs in humans with careful animal model choice and good experimental methodology.

The normal rat model was used to detect the interaction instantly, whereas the diabetic rat model was used to authorize the same reaction in the drug's actual usage condition [241, 242, 279]. Aside from the ease of serial blood sampling design, several studies have shown functional similarities between CYP forms in rabbits and humans [280, 281], showing that the rabbit is a suitable in vivo model for PK drug interactions. As a result, a rabbit model, a different species, undertook PK and PD tests to confirm the presence of interaction. If rat and rabbit species occur with an interaction, it is also expected to occur in humans, given their representational inconsistency with humans [241, 242, 279].

Based on this context, the purpose of this designed study is i) to explore the effect of piperine on chosen antidiabetic drugs (repaglinide and sitagliptin) by PD interaction investigations in rats (normal and streptozotocin-induced diabetes) and rabbits (normal and streptozotocin-induced diabetes) and PK interactions investigations in rabbits (normal and streptozotocin-induced diabetes), ii) To provide the information regarding the mechanism(s) of interaction(s), If an interaction occurs.

Insulin is the major hormone influencing glucose metabolism, a key aspect of metabolic balance. Insulin regulates glucose storage and use and helps to maintain glucose homeostasis. Glucose, which is assessed metabolically, controls insulin activity. Diabetes is characterized by a loss of glucose homeostasis and increased plasma glucose levels. Insulin resistance (IR) seems to be a situation in which normal or higher insulin levels result in a diminished biological response [282], which relates to insulin-mediated glucose clearance sensitivity. Considering this perspective, the study was designed to determine glucose levels (by GOD-POD method) [246] and insulin levels (by ELISA method) [247] as PD parameters at specified interval days in

animal models to evaluate the influence of piperine activity with repaglinide and sitagliptin to illustrate the clinical implications in patients with diabetes.

The study in rats (normal and streptozotocin-induced diabetes) and rabbits (normal and streptozotocin-induced diabetes) was stretched for twenty-one days to investigate the effects of repaglinide and sitagliptin drugs on piperine activity concerning blood glucose and insulin levels on day 1 (single-dose study) and days 3, 7, 14, and 21 (multiple-dose study). On the other hand, the research was extended in rabbits (normal and streptozotocin-induced diabetes) for 21 days at defined intervals to explore the effects of the chosen repaglinide and sitagliptin on piperine activity in terms of PK parameters on day 1 and day 21.

Blood samples were drawn by retroorbital puncture in rats [248] and marginal ear veins in rabbits [250] since the studies were reported to be effective techniques for collecting tiny amounts of blood. DM was induced with streptozotocin [251, 283] because it was less expensive and more readily accessible.

Further this research was planned i) by inferring the oral dosages of investigational drugs in rats and rabbits based on a literature review that highlights the clinical relevance, ii) to perform PD and PK experiments in the same rabbit group at the same time in order to ascertain a clear correlation between PD and PK and to investigate potential drug interaction mechanisms, iii) to perform single-dose and multiple-dose PK/PD interaction research in order to give useful information on the time course and amplitude of piperine with repaglinide and sitagliptin interactions with respect to pre-clinical perspective, iv) to detect repaglinide and sitagliptin plasma concentrations in rabbits using a simple, accurate, and validated HPLC technique, v) to use WinNonlin tool to do noncompartmental analysis on the repaglinide and sitagliptin concentration data and vi) For statistical analysis, the PD findings were exposed to one-way ANOVA followed by Tukey's test, while the PK results were exposed to Student's paired t-test.

Rats and rabbits are reported to be more sensitive to repaglinide and sitagliptin responses. As a result, we performed dose-effect-relationship research on repaglinide

and sitagliptin to determine the oral dosage that results in a 35% decrease in blood glucose levels in rats.

Repaglinide has been shown to have hypoglycemic/antihyperglycemic action through both pancreatic (raising insulin production by inhibiting K+ channels in pancreatic cells) and extrapancreatic (escalating glucose absorption by tissues) mechanisms [284]. In single-dose and multiple-dose studies, repaglinide produced maximum percent blood glucose reduction, the maximum insulin level in rats and rabbits (normal and streptozotocin-induced diabetes), and maximum plasma concentration in rabbits (normal and streptozotocin-induced diabetes), representing the consistency between PD and PK of repaglinide. Sitagliptin is known to yield antihyperglycemic activity by GLP-1 production to release more insulin levels. In single and multiple-dose studies, sitagliptin produced maximum blood glucose reduction and maximum insulin level in diabetic rats and rabbits, but no significant changes in normal rats and rabbits, and made maximum plasma concentration in rabbits (normal and streptozotocin-induced diabetes) in a single-dose and multiple-dose studies, representing PD and PKs of sitagliptin.

Piperine alone demonstrated antihyperglycemic action in diabetic rats and rabbits, indicating a change in blood glucose and insulin levels. Piperine alone has no hypoglycemic effect in normal rats and rabbits, indicating no significant changes in blood glucose and insulin levels.

In rats (normal and streptozotocin-induced diabetes) and rabbits (normal and streptozotocin-induced diabetes), piperine significantly ($p<0.01$) decreased blood glucose levels, significantly ($p<0.01$, $p<0.05$) increased percent blood glucose reduction, and significantly ($p<0.05$) raised insulin levels when treated along with repaglinide. In rats and rabbits, the anti hyperglycemic impact is greater in diabetic rats and rabbits than the hypoglycemic effect in normal rats and rabbits. Piperine's efficacy in exacerbating DM is demonstrated in this study.

In comparison to SDT, MDT significantly increased repaglinide activity. The substantial impact of single and multiple doses of piperine with repaglinide may be

attributed to a more synergistic effect on β-cells to release more insulin in the multiple-dose study than in the single-dose study.

Piperine and repaglinide have a significant PD interaction in animal models (rats and rabbits) due to the synergistic effects of piperine and repaglinide on glucose and insulin levels. Because this interaction existed in two different species, it is probable to arise in humans as well, which may result in enhanced repaglinide activity. Consequently, clinicians should monitor combo in a clinical setting.

Subsequent single-dose and multiple-dose treatment of repaglinide with piperine in rat and rabbit models, repaglinide PKs were significantly ($p<0.05$) improved, confirming the presence of a possible interaction between piperine and repaglinide. Multiple-dose repaglinide treatment with piperine dose treatment resulted in a greater enhancement than a single-dose study.

The PK properties of repaglinide changed significantly ($p<0.05$). In rabbits (normal & streptozotocin-induced diabetic), an apparent rise in AUC of repaglinide in the presence of piperine was due to augmented C_{max}, decreased CL, and increased $T_{1/2}$ (tables 7.15 & 7.18). Piperine inhibited the CYP3A4 metabolic enzyme, causing repaglinide metabolic inhibition.

Piperine's pharmacological interaction with repaglinide was thought to occur at hepatic locations. According to structural and biochemical investigations, piperine inactivates CYP3A4. Piperine inhibits enzymes, which has significant therapeutic implications when drug metabolism is decreased, resulting in greater drug concentration and bioavailability of repaglinide. Piperine's ability to inhibit enzymes is due to its structure. The 7-methoxycoumarin-Odemethylase (MOCD) activities and aryl hydrocarbon hydroxylase (AHH) are inhibited by the methylenedioxyphenyl (MDP) ring, its side chain, and piperidine moiety. Alteration of just one component in the piperine molecule can cause differential inhibition of the two kinds of monooxygenase activity [268]. Other proposed piperine inhibitory methods include the development of an intermediate metabolic complex (MIC) [285], competitive [286], or mixed competitive-noncompetitive CYP3A4 inactivation.

Piperine exhibits a significant PK interaction with repaglinide in animal studies owing to CYP3A4 inhibition at the metabolic level. The current study data showed that piperine significantly increased the bioavailability of repaglinide in both single-dose and multiple-dose studies. Because the study showed the interaction in rabbits, it is likely to occur in people as well, resulting in increased repaglinide activity and the need for dose modifications. As a result, when this combination is recommended for therapeutic benefit, caution should be exercised.

In diabetic rats and rabbits, piperine significantly ($p<0.001$, $p<0.01$) dropped blood glucose levels (tables 8.4 & 8.10), significantly ($p<0.001$) increased percent blood glucose reduction (tables 8.5 & 8.11), and significantly ($p<0.05$, $p<0.01$) augmented insulin levels of sitagliptin (tables 8.7 & 8.13), while there were no significant changes observed in glucose levels (tables 8.2 & 8.8), percent blood glucose reduction (tables 8.3 & 8.9) and insulin levels (table 8.6 & 8.12) in normal rats and rabbits. The antihyperglycemic impact of sitagliptin in rats and rabbits is greater in diabetic rats and rabbits, but no hypoglycemic activity was observed in normal animals (rats and rabbits). Piperine's efficacy in exacerbating DM is demonstrated in this study.

In diabetic rabbits, multiple-dose therapy significantly enhanced sitagliptin activity compared to single dosage treatment. The significant effect of single and multiple-doses of piperine with sitagliptin may be attributed to a greater additive effect in which piperine influences β-cells and sitagliptin influences GLP-1 to release more insulin in the multiple-dose study compared to single-dose study.

Piperine and sitagliptin have a significant PD interaction in diabetic animals (rats and rabbits) due to the additive effects of piperine and sitagliptin on glucose and insulin levels. And no significant changes were observed in normal animals (rats and rabbits). Because this interaction was found in two different species, it is probable to occur in humans as well, resulting in significantly enhanced sitagliptin activity. As a result, this combination therapy should be monitored in a clinical state.

In rat and rabbit models, sitagliptin PKs were considerably ($p<0.05$) improved following single-dose and multiple-dose treatment with piperine, confirming the

presence of a possible interaction between piperine and sitagliptin. The effect was greater with multiple-dose therapy of sitagliptin with piperine.

There was a significant ($p<0.05$) variation in sitagliptin PK parameters. In rabbits (normal & streptozotocin-induced diabetic), an increased Cmax decreased clearance and increased $T_{1/2}$ all led to an apparent rise in AUC of sitagliptin in the presence of piperine (table 8.15 & 8.18). The study results might be due to piperine's major P-gp inhibition and partial CYP3A4 inhibition of sitagliptin.

Sitagliptin does not undergo extensive metabolism, even though the CYP3A4 and CYP2C enzymes are metabolic enzymes of sitagliptin. Piperine was expected to interact with sitagliptin mostly by P-gp inhibition, which reduces sitagliptin efflux transport and partially through CYP3A4 suppression. According to structural and biochemical investigations, piperine is a time-dependent inhibitor of CYP3A4. Piperine inhibits enzymes, which has significant therapeutic implications since it reduces drug metabolism, increasing drug concentration and sitagliptin bioavailability. The structure of piperine is responsible for its capacity to inhibit enzymes. The methylenedioxyphenyl (MDP) ring, its side chain, and piperidine moiety all work together to block 7-methoxycoumarin-Odemethylase (MOCD) and aryl hydrocarbon hydroxylase (AHH) activities. A single change to the piperine molecule can result in differential suppression of the two types of monooxygenase activity [263]. Other proposed piperine inhibitory methods include development of a metabolic intermediate complex (MIC) [285], competitive [286], or mixed competitive-noncompetitive CYP3A4 inactivation [287]**.** However, in the event of a pharmacological interaction between sitagliptin and piperine, piperine inhibited CYP3A4 partially, whereas P-gp inhibited favourably.

Piperine has a strong PK interaction with sitagliptin, mostly at the P-gp efflux transporter level suppression and partially at the metabolic level via CYP3A4 suppression in animal studies (rats and rabbits). Piperine significantly improved the bioavailability of sitagliptin in both single-dose and multiple-dose studies, according to the shown data. Because of the interaction demonstrated in rabbits, it is probable to occur in humans as well, resulting in enhanced sitagliptin activity that may necessitate

dose modification. As a result, caution should be exercised when this combination is suggested for therapeutic benefit.

9.2. Conclusion

- There is a significant PD interaction between Repaglinide and Piperine in normal and diabetic animal models (rats and rabbits) resulting in the synergistic effects on glucose and insulin levels. Combination of Repaglinide with Piperine showed significant anti hyperglycemic action in diabetic animals (rats and rabbits) and significant hypoglycemic impact in normal animals (rats and rabbits).

- There is a PK interaction of Repaglinide with Piperine also and significant effect was observed on bioavailability of repaglinide (rabbits). Piperine increased the bioavailability of repaglinide in normal and diabetic rabbits. The increased plasma concentration levels of repaglinide in the presence of piperine could be because of its diminished metabolism caused by CYP3A4 inhibition, which slowed the elimination activity, as evidenced by a significant decrease in repaglinide elimination and clearance from PK parameters. Inhibition of CYP3A4 may be responsible for PK interaction between repaglinide and piperine.

- As these interaction was observed in two dissimilar species, it indicates the possibility of similar interaction in humans as well, necessitating dose adjustments. As a result, this combination should be closely watched in a clinical setting and caution should be used when this combination is suggested for therapeutic benefit.

- There is a significant PD interaction of Sitagliptin with Piperine in diabetic animal models (rats and rabbits) but not in normal animals (rats and rabbits). The study showed a significant anti-hyperglycemic action in diabetic animals (rats and rabbits) but no hypoglycemic impact in normal animals (rats and rabbits) when sitagliptin was co-administered with piperine.

- There is a PK interaction of Sitagliptin with Piperine also and piperine increased the bioavailability of sitagliptin. MDT of sitagliptin had a greater effect on piperine activity (plasma concentration level and PK) than SDT in diabetic rabbits. The

increased plasma concentration of sitagliptin when co-administered with piperine may be attributable to its decreased metabolism via CYP3A4 inhibition and reduced clearance via P-gp efflux transporter inhibition.

- Despite increase in Sitagliptin exposure, no significant variation in PD parameters was observed in normal rabbits. Sitagliptin's mechanism of action may be held responsible for this. There could be several reasons for this like saturation of target receptors or activation of compensatory mechanisms to maintain homeostasis.

- Extrapolation of results to humans require studies at large scale. However, as piperine increased the bioavailability of Repaglinide and Sitagliptin; caution should be taken in hypoglycemia patients when this combination is suggested for therapeutic benefit.

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LIST OF ORIGINAL PUBLICATIONS

S. No.	Title of paper with author names	Name of journal/conference	Published date	ISSN no/ vol no, issue no	Indexing in Scopus/ Web of Science/ UGC-CARE list (*please mention*)
1.	Sagar Pamu, Sazal Patyar, Lakshmi Thakkalapally. Development and Validation of a Novel RP-HPLC Analytical Method for Sitagliptin in Human Plasma	Journal of Pharmaceutical Research International	31-08-2021	2456-9119, 33, 42B	Web of Science
2.	Sagar Pamu, Thakkalapally L, Patyar RR, and Patyar S. Influence of Piperine on Pharmacodynamics and Pharmacokinetics of Repaglinide in Rabbits	International Journal of Biology, Pharmacy, and Allied Sciences	01-09-2022	2277-4998	Web of Science
3.	Sagar Pamu, Sazal Patyar, and Lakshmi Thakkalapally. Sitagliptin with Piperine Pharmacodynamic and Pharmacokinetic Studies in Normal and Diabetic Rabbits.	Current Drug Therapy	Accepted in July 2023	1574-8855	Scopus & Web of Science

LIST OF PAPER PRESENTATIONS

S. No.	Title of paper with author names	Name of conference	Held on	Oral/ Poster Presentation	Organized by
1.	P. Sagar, Method Development and Validation of Repaglinide by using RP-HPLC	3rd National Conference on Artificial Intelligence: A Deeper Insight Towards Healthcare and Pharma Industry PHARMA CAPSTONE-2K20	Conference Held on 7th March 2020	Oral	KVK College of Pharmacy
2.	P. Sagar, A Review on Herbal Product Piperine, its Pharmacokinetic Drug Interactions with Various Drugs	4th International Conference on Innovations in Pharmaceutical Sciences	Conference held on 9th & 10th August 2019.	Oral	Guru Nanak Institutions Technical Campus-School of Pharmacy
3.	Sagar Pamu, Influence of Piperine on Pharmacodynamics of Repaglinide with Respect to Blood Glucose & Insulin Levels in Rabbits	25th National Conventional of Society of Pharmacognosy and International Conference on New Horizons of Natural Products and Ayush Remedies	Conference held on 27-28 November 2021.	Oral	Graduate School of Pharmacy, GTU, and Society of Pharmacognosy

BOOK PUBLICATION

S. No.	Title of e-Book	Author Names	Published date	ISBN
1.	Piperine with Antidiabetic & Other Synthetic Drugs	Sagar Pamu, Sazal Patyar	16th April 2019	978035959556-3

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1.	Piperine Mediated Pharmacokinetic Mechanisms	Sagar Pamu, Sazal Patyar, Lakshmi Thakkalapally	6 April 2023	A-145014/2023