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IMPROVING BLOOD GLUCOSE REGULATION ON ALLOXAN MONOHYDRATE-INDUCED DIABETIC MICE: A SYNERGISTIC EFFECT OF PIPERINE AND LINAGLIPTIN

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ABSTRACT

Aim: High blood sugar levels are the outcome of diabetes mellitus, a metabolic and chronic condition caused by insufficient insulin secretion, action, or both. The main purpose of this study is to evaluate the effect of piperine with linagliptin on the lowering of blood glucose levels in alloxan- induced diabetic mice through the estimation of blood glucose levels and the mean plasma concentration of linagliptin. **Methods:** Based on randomization technique, Alloxan-induced(150mg/kg,i.p.) thirty two CD-1 Male and Female Mice (aged 8-9 weeks old)were divided into 4 groups, Group 1(Disease control)can be administered with 0.5% CMC, Group 2(Drug alone) animals of both sexes male and female were administered with Linagliptin(1mg/kg,p.o.) and Group 3(Therapeutic dose) in this all the animals were administered with both piperine and linagliptin (10mg/kg, p.o.+1mg/kg,p.o.) and Group 4(Sub-therapeutic dose) in this all the animals were administered with both piperine and linagliptin (10mg/kg, p.o.+0.3mg/kg, p.o.). Blood glucose levels were estimated at day 1,7 and 14. On the 14th day of treatment the animal were anaesthetized, blood samples were collected through the saphenous vein method and plasma were separated for the estimation of mean plasma concentration of linagliptin. **Results:** The findings of this study are that a combination of piperine with linagliptin therapeutic dose (10 mg/kg + 1 mg/kg,p.o.) showed significantly higher lowering of blood glucose levels as compared to

linagliptin alone (1 mg/kg, p.o.) and also the Disease control groups on both the 7th and 14th days ($p<0.05$). while piperine in combination with the linagliptin sub-therapeutic dose (10 mg/kg + 0.3 mg/kg, p.o.) showed significantly higher lowering of blood glucose levels as compared to the disease control group as well as with the linagliptin alone group (1 mg/kg, p.o.). Then, the pharmacokinetic profiles of linagliptin in mice after oral administration of linagliptin alone(1mg/kg,p.o.) and in combination with piperine (10mg/kg,p.o.) were determined using a validated LC-MS/MS method. The results showed that substantial increase in bioavailability when piperine is combined with linagliptin. **Conclusion:** From the results of this experiment, we showed that piperine inhibits both the drug transporter p-gp and the major drug metabolizing enzyme CYP3A4. piperine has the potential to be used as a bioenhancer in combination with linagliptin, which can help reduce the adverse effects, dosage of linagliptin and cost of linagliptin.

KEYWORDS: Diabetes, Bio-enhancingeffect, piperine, Linagliptin, bioavailability, AUC, Cmax, $t_{max,}$ LC-MS/MS, Blood glucose levels, mean plasma concentration of linagliptin.

Highlights

- Co-administration of piperine with linagliptin attenuates alloxan-induced high blood glucose levels in mice.
- The combined use of piperine with linagliptin demonstrated an enhanced protective effect against alloxan-induced diabetic mice.
- PIPERINE inhibited the CYP-3A4 and P-gp enzymes
- Piperine enhanced the plasma concentration and AUC of linagliptin

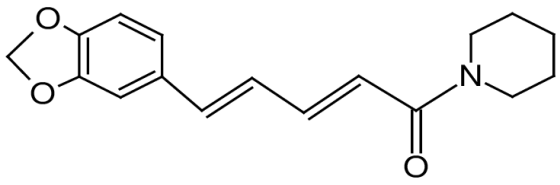
Abbreviations: Diabetes Mellitus(DM), Type 2 Diabetes Mellitus(T2DM), Insulin Resistance(IR), Free fatty acids(FFR), Latent autoimmune Diabetes in adults (LADA), Maturity Onset Diabetes of Young (MODY), Type 1 Diabetes Mellitus(T1DM), Insulin-dependent Diabetes mellitus(IDDM), Gestational Diabetes Mellitus(GDM), Double Diabetes (DD), Anti-Diuretic Hormone(ADH), Neonatal Diabetes Mellitus(NDM), Continuous glucose monitor(CGM), p-glycoprotein(PGP), Glucagon like peptide inhibitors(GLP), Sodium glucose transporter protein-2(SGLT2), Di-peptidyl peptidase inhibitors(DPP4), Uridine diphosphate(UDP), Glucose dependent insulinotropic polypeptide(GIP), Pharmacokinetics(PK), Pharmacodynamics (PD), Mean Residence time (MRT), Carboxy Methyl Cellulose(CMC), Reactive oxygen species(ROS).

INTRODUCTION

Diabetes mellitus (DM) is a set of metabolic illnesses defined by chronic hyperglycemia caused by a disruption in insulin secretion, function, or both.[1,2] Diabetes-related chronic hyperglycemia is linked to long-term harm, dysfunction, and failure of many organs, particularly the heart, blood vessels, kidneys, nerves, eyes, and kidneys.[3] Following that, persistent hyperglycemia directs the development of diabetes-related problems by causing epigenetic changes in target cells, lowering patient's quality of life[4,5] and placing a significant cost on the health systems of all nations.[6] Type 2 diabetes (T2D) is the most frequent clinical manifestation of diabetes, accounting for 90% of cases.[7] Diabetes is becoming one of the most serious public health issues.[8,9] and the fifth largest cause of mortality worldwide.[10] DM affects around 463 million people worldwide.[11] It is predicted that this figure will rise to 700 million by 2045.[12,13] Ethiopia is one of the first four countries in Sub-Saharan Africa (SSA) to have a higher percentage of diabetics (5.2%).[14] Diabetes was initially described by the Egyptians and is characterized by weight loss and polyuria. The term diabetes mellitus (DM) was coined by the Greek physician Aertaeus.[15] Diabetes means "to pass through" in Greek, while mellitus is the Latin word for honey (which refers to sweetness). Diabetes is a leading cause of long-term illness and premature death, claiming more lives each year than HIV/AIDS, with nearly one death every 10 seconds.[16]

Most pharmacokinetic processes depend heavily on drug absorption, which is the initial phase that has a significant impact on a medication's bioavailability. The most popular and preferred method of administration is oral. Due to its enormous surface area, the small intestine is the primary site of medication absorption when taken orally. Numerous drug and patient characteristics influence the rate and degree of drug absorption across the gut barrier.[17,18] It is still a serious clinical concern that many critical medications that are administered orally have low oral bioavailability.[19] Depending on the reasons that restrict a drug's absorption and metabolism, there are many ways to address low oral bioavailability.[20–25] As a result, the term "bioenhancer" was coined. Bioenhancers are compounds that, by themselves, are not therapeutic entities, but when combined with an active medicine, they increase the drug's pharmacological efficacy.[26] Bioenhancers are substances that can increase the oral bioavailability of drugs by modulating their absorption, metabolism, or transport.[27–31] Bioenhancers are chemicals that are frequently employed in combination therapy to boost a drug's bioavailability or biological activity. Plant-derived biomolecules or their semi-synthetic derivatization have increased the manufacture of pharmaceuticals.[32]

Piperine is one of the most widely used natural bioenhancers, as it can enhance the bioavailability of various drugs and nutrients by modulating their absorption, metabolism, or transport in the gastrointestinal tract.[31] Bioenhancers are used for a variety of known reasons, including their nontoxicity, effectiveness at low concentrations, and straightforward formulation procedures.[33] Hans Christian Qrsted made the discovery of piperine (PIP) in 1819.[34]



Piperine

Piperine (1-piperoyl piperdine) is one of the primary components of the Piper species(35). Piperine, the trans-trans-isomer of 1-piperonylpiperidine, is present in both black pepper (Piper nigrum) and long pepper (Piper longum), both of which are members of the piperaceae plant family. Black pepper is a flowering Piperaceae vine farmed for its fruit, which is frequently dried and used as a spice and condiment.[36–39] The solid form of piperine, often referred to as 1-peperoyl piperidine, has a melting point of 128 °C, is optically inert, and only sporadically dissolves in water with cis-trans isomerism.[40–46] Black and long peppers (Piper nigrum and longum) are spices that are often used in cooking. Piper species are also commonly used in Ayurvedic remedies.[41] It has been shown that piperine increases the bioavailability of several drugs and other pharmacologically active substances, including curcumin, resveratrol, epigallocatechin-3-gallate, and theophylline, in studies including both animals and human volunteers.[47–50] The principal active element, piperine, which was identified as the first bioenhancer in 1979, is present in both black pepper (Piper nigrum Linn.) and long pepper (Piper longum Linn.).[51,52] Piperine can block P-glycoprotein, another significant efflux transporter found in the hepatobiliary system and in enterocytes. The potential of piperine to improve the bioavailability of various medications and nutrients by increasing their absorption and decreasing their metabolism, however, is one of its most impressive properties. The term "bioenhancement" or "bioavailability enhancement" refers to this occurrence. Piperine has been demonstrated to increase the bioavailability of several medications, including rifampicin, curcumin, propranolol, carbamazepine, ciprofloxacin, metronidazole, and many others, making it one of the most extensively investigated

bioenhancers. Additionally, it has been claimed to increase the absorption of various minerals, including vitamin B6, selenium, beta-carotene, and amino acids.[53–56]

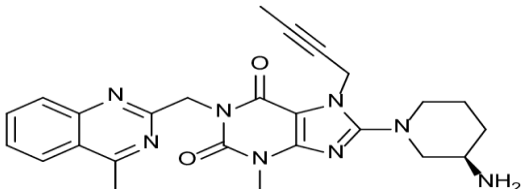
1.1. Mode of action of piperine as a bioenhancer

Although the exact method of action of piperine as a bioenhancer is unknown, some potential mechanisms include

- **By Increasing solubility:** Piperine may promote the production of micelles, which are tiny molecular clusters that aid in the digestion of lipids and medications that are lipid-soluble.
- **By Enhancing permeability:** Piperine might modify the lipid environment and membrane dynamics of the intestinal cells, which would increase their susceptibility to medicines and nutrients.
- **By Impeding metabolism:** Piperine can prevent cytochrome P450, glucuronidase, sulfotransferase, and glycoprotein from breaking down medicines and nutrients. This might boost the amount of medicine that reaches the systemic circulation and prevent drug action from being lost.
- **By modulating transporters:** P-glycoprotein(P-gp), multidrug resistance protein, organic anion transporting polypeptide, and organic cation transporter are a few of the transporters that are involved in the uptake and efflux of drugs and nutrients across intestinal cells. Piperine may affect their expression and function.[57–63]

DPP-4 inhibitors are a unique pharmacological class of glucose-lowering medicines that open up new avenues for the treatment of type 2 diabetes (T2DM).[64] These substances raise the active levels of incretin hormones. This is accomplished by inhibiting the DPP-4 enzyme, which rapidly destroys glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). The mode of action of DPP-4 inhibitors are unique from any other class of oral glucose-lowering medicines now on the market.[65–69] Linagliptin has been demonstrated to have more potency, selectivity, mechanism, and duration of effect than other DPP-4 inhibitors that are currently on the market.[70] In several recent reviews, the primary pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of linagliptin as well as the most significant outcomes attained with linagliptin in the therapy of patients with T2DM have been disscused in detail.[71–73]

1.2. Treatment for type 2 diabetes: It includes the use of the drug linagliptin together with a healthy diet and regular exercise. It is a member of a group of medicines known as dipeptidyl peptidase-4 (DPP-4) inhibitors, which function by raising the levels of specific hormones that assist in controlling blood glucose levels.[74–76] In the United States, linagliptin is sold under the brand names Tradjenta and Trajenta, respectively.[77–79] In contrast to other medications in the same class, linagliptin has a complex chemical structure with a xanthine base. The chemical formula of linagliptin is $C_{25}H_{28}N_8O_2$, and its molecular weight is 472.553 g/mol.[80–83]



Linagliptin

Linagliptin has a limited oral bioavailability, with a range of 29.5% to 30%(84). This indicates that only a limited portion of the medication administered orally enters the systemic circulation. The first-pass metabolism of linagliptin in the intestine and liver, as well as its efflux by the transporter protein P-glycoprotein (P-gp), are the main causes of its low oral bioavailability.[85]

However, only a small portion of linagliptin, which has a low oral bioavailability of roughly 30%, enters the bloodstream after being ingested. This could reduce its therapeutic effectiveness and make bigger doses necessary to have the intended effect.[86–88]

Piperine, a naturally occurring substance included in long and black pepper, has been proven to increase the bioavailability of a number of medications, including linagliptin. Piperine may work by preventing the cytochrome P450 and glucuronidase enzymes from metabolizing medicines in the liver and intestine. As a result, there is a decrease in medication breakdown and an increase in drug uptake and circulation. Additionally, piperine may make the intestinal membrane more permeable, allowing more medicines to flow through.

Therefore, improving the oral bioavailability of linagliptin through the use of piperine as a bioenhancer may enhance its pharmacological effects. Patients with type 2 diabetes mellitus may benefit from lower doses, fewer side effects, and improved glycemic control, and improve patient's quality of life as a result of this.[89–93] As far as we know, there has never

been a report in the literature on the effects of piperine on the pharmacokinetics and antihyperglycemic properties of linagliptin. Considering this, the current investigation was carried out to describe the effects of piperine on the pharmacokinetics and anti-hyperglycemic properties of linagliptin in alloxan-induced diabetes.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals: Alloxan Monohydrate was purchased from Sisco Research Laboratories Pvt ltd. (Hyderabad), Carboxy methyl cellulose was obtained from sigma Aldrich, Piperine(API) was purchased from Carbanio(Hyderabad), Linagliptin(API) was purchased from Vivan life sciences(Hyderabad), and Glucose estimation kit(On call)was purchased from their respective representatives. Acetonitrile and methanol were purchased from sigma Aldrich, and all other chemicals obtained from Biolivz Research Center were of reagent grade and all the solvents used were of High performance liquid chromatography (HPLC) grade.

2.2 ANIMALS: Thirty-two CD-1 Male and Female Mice (aged 8-9 weeks old) with body weights of 25-35 g were obtained from Hylasco Labs Hyderabad. The animals were kept (4/cage) under controlled conditions (temperature of 22 ± 2ºC), relative humidity of 55±5%, and 12 hours of light and dark cycles. The animals were maintained on a standard and protein-rich diet with free access to water. All the animals were acclimatized for a period of 7 days under the above conditions **(PD-BRC-23-001)** at Biolivz Research Center Private Limited (Polampally). This study protocol was approved by the Institutional Animal Ethics Committee (IAEC). All of the animals were chosen for the experiment based on their body weight and blood glucose levels, using randomization approaches.

2.3 Drug preparation for animal experiment

To keep the solvent uniform, piperine will be administered orally and suspended in 0.5% CMC. Then, the appropriate drug solutions were made.[94]

2.4 Diabetic Induction

After an overnight fast, mice were given a single dosage of alloxan monohydrate, dissolved in normal saline (150 mg/kg body weight, i.p.). Following an hour of alloxan administration, the animals were given standard pellets and ad libitum access to water. The animals were stabilized for a week, and those with moderately high blood sugar levels (measured by a glucometer, between 250 to 350 mg/dl) were chosen for the study.[95–97]

2.5 Experimental Design

Thirty-two CD1 mice of both sexes containing diabetes were randomly divided into four groups with each group containing eight animals(n=8)

Group-1: Disease Control group received (0.5% CMC, p.o.)

Group-2: Linagliptin alone group received (1mg/kg,p.o.)

Group-3: Therapeutic dose group Piperine(10mg/kg,p.o.)+Linagliptin(1mg/kg,p.o.)

Group-4: Sub-therapeutic dose group Piperine(10mg/kg,p.o.)+Linagliptin(0.3mg/kg,p.o.)

An identification mark was given to the mice of each group using distinctive tail markings. Each mouse was weighed, and the doses were calculated accordingly.

2.6 Experimental Protocol

The total study lasted for 21 days. All groups of animals were induced with a single dose of alloxan monohydrate (150mg/kg body weight, i.p.) After the animals have been randomized, they can be divided into particular groups for further study. Randomization technique used for selecting animals based on their size and blood glucose levels. Randomization is a technique used in animal studies to avoid bias and ensure that underlying variables do not cause skewed data for each experimental group(98–102). All animals were weighed prior to the experiment to ensure for the accurate dosing of study agents. Group 1(Disease control)can be administered with 0.5% CMC, Group 2(Drug alone) animals of both sexes male and female were administered with Linagliptin(1mg/kg,p.o.) and Group 3 which is nothing but therapeutic dose group in this all the animals were administered with both piperine and linagliptin (10mg/kg, p.o.+1mg/kg,p.o.) and Group 4 which is nothing but Sub-therapeutic dose group in this all the animals were administered with both piperine and linagliptin (10mg/kg, p.o.+0.3mg/kg, p.o.) for 14 days after the induction of alloxan monohydrate and the animals were stabilized for a week after the administration of alloxan monohydrate(i.e. from day 1 to day 7). The dose levels of piperine have been selected based on the previous studies with piperine.[103–105] and the linagliptin dose levels have been selected based on the fda approved pharmacological review data of linagliptin.[106–108] From day 8 which means after the induction of alloxan monohydrate that means here day 8 is considered as a day 1 for administration of treatment drugs, so here from day1 to day14 Body Weight, Food intake and Blood Glucose Levels were estimated by using the glucometer. Here Group 3 and 4 were co-administered with piperine, so here the piperine can be given first and followed by linagliptin. All animals were exposed only once to every experiment.

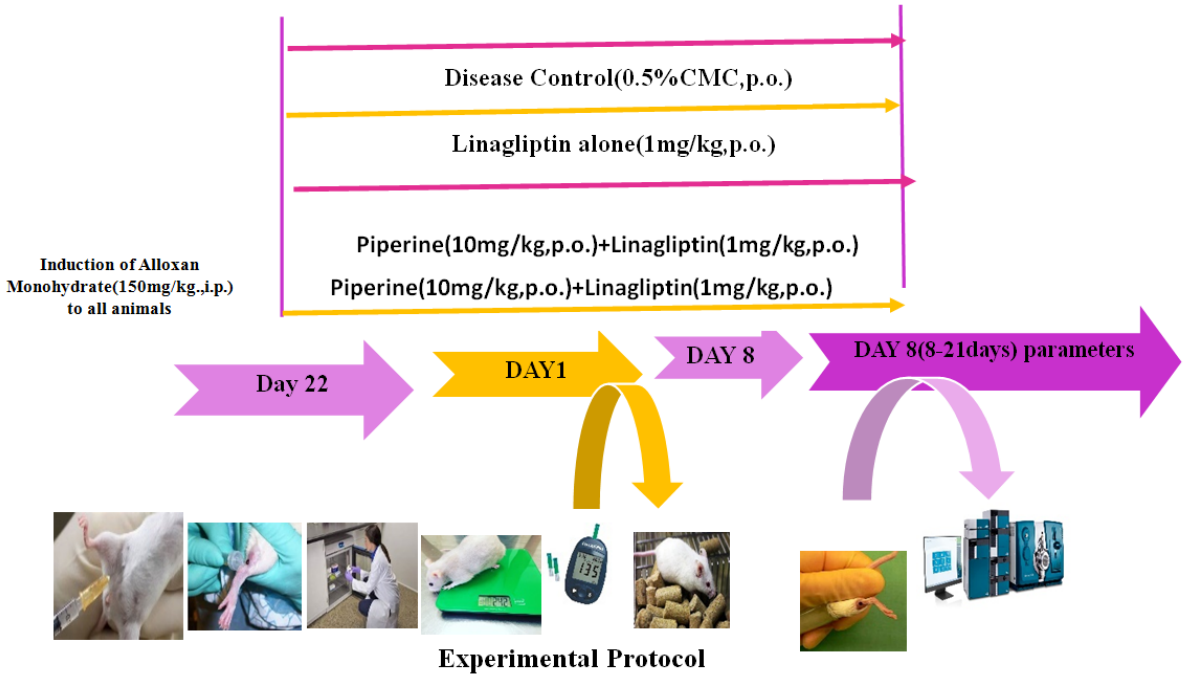


Fig. 1: Scheme representing experimental protocol and intervals for various parameters.

2.7 Procedures for estimating Body Weight, Food intake and Blood Glucose levels

2.7.1 Body Weight

Monitoring the body weight of mice is crucial in diabetes research as it provides valuable insights into their health status and the progression of disease. In this study weigh the animals by using weighing balance daily before administering the drug and record their body weights. And this data is used for accurate dose calculations. During weighing process ensure a calm and comfortable environment, handle them gently, and use appropriate equipment to ensure accurate measurements. And by this way, we can minimize stress and ensure the well-being of animals during the weighing process.[109–112]

2.7.2 Food Intake

Food Intake level is a crucial parameter in a diabetes. It helps to determine the impact of diet on diabetes progression and management. In this study daily can measure food consumption by providing 40 grams of food to each cage at consistent timings. Afterwards, Record the amount of food left and calculate the amount they should have consumed. By doing this we can evaluate how different diets influence blood sugar levels and overall health in mice. And this might provides insights into the relationship between the diet and diabetes. And while doing this we can ensure that the weighing balance is properly calibrated and handle the food with clean utensils to avoid contamination. It's important to maintain consistency in feeding

times and provide a controlled environment to minimize any external factors that may affect food intake.[113–118]

2.7.3 Blood Glucose Levels

Estimating Blood Glucose levels is very much important for managing Diabetes because it helps monitor and control Blood Sugar Levels. To estimate Blood Glucose Levels in mice, we can use a Glucometer, and then collecting a small blood sample, usually from the tail vein, and applying it to the test strip of the glucometer. The glucometer then provides a reading of the blood glucose level. It's important to follow the instructions provided with the glucometer and handle the mice with care during the procedure.

To ensure accurate blood glucose measurements, it's important to follow these measures:

- Use a clean and sterile lancet to minimize the risk of infection
- Handle the mice gently to minimize stress and discomfort.
- Choose an appropriate site for blood collection, such as Tail vein
- Make sure the mice are properly restrained during the procedure.
- Follow the instructions provided with the glucometer for accurate testing
- Record the blood glucose levels at consistent intervals for monitoring and analysis.
- And finally, Remember, it's essential to prioritize the well-being and comfort of the mice throughout the process.

While collecting blood samples from the tail vein, It's important to take proper care. Ensure the tail is clean and dry before the procedure. use a sterile lancet to make a small incision, being careful not to cause excessive bleeding. Apply gentle pressure to the area after collecting the blood sample to promote clotting. And we can use spirit to clean the tail before estimating blood glucose levels in mice. it helps to ensure a clean and sterile site for blood collection. Just make sure to use a small amount and allow it to dry completely before proceeding with the procedure. Finally, Monitor the tail for any signs of infection or discomfort in the following days.[119–121]

2.7.3.1 Steps to estimate blood glucose levels using the On Call Plus glucometer

Wash your hands with soap and water and dry them thoroughly.

1. Insert a test strip into the glucometer.
2. Use the lancing device to prick your finger and collect a small drop of blood.

3. Touch the test strip to the blood sample and wait for the glucometer to display your blood glucose level.[122–124]

2.8 Pharmacokinetic study

Mice were randomly divided into 2 groups (3 mice per group).one group received a linagliptin alone(1mg/kg,p.o.) and the other group received a co-administration of piperine(10mg/kg,p.o.) with linagliptin(1mg/kg,p.o.). And on the 1stday of giving treatment the above 2 groups (i.e, group-2 and 3) of animals blood samples(300µl each)were collected by an saphaneous vein route at 30min, 1hr, 3hr, 5hr, 7hr and 24h. The blood samples were centrifused and the obtained plasma was stored at -20ºC until analyzed. And also on the 14th day of giving treatment the same groups of animals blood samples (300µl each) were collected by an saphaneous vein route at 30min, 1hr, 3hr, 5hr, 7hr and 24h. The blood samples were centrifused and the obtained plasma was stored at -20ºC until analyzed. All animals were exposed only once to every experiment.

2.8.1 LC-MS/MS Method for Quantification of Linagliptin in Mice Plasma

Analysis: The analysis of linagliptin in plasma samples collected from mice was conducted on an Exion LC UHPLC system hyphenated with a Sciex Triple Quad^{TM} 4500 mass spectrometer. Chromatographic separation was achieved using an XBridge C18 (2.1 × 50 mm, 3.5 µm) column maintained at 50 °C. Mobile phase consisting of 0.1% v/v formic acid in water (A) and 0.1% v/v formic acid in acetonitrile (B) were used to elute linagliptin and alogliptin (internal standard) under gradient conditions. The mass spectrometer equipped with an Iondrive^{TM} Turbo V ionization source interface operated in positive ion selected reaction monitoring (SRM) mode was used for detection. SRM transitions of m/z 473.3 → 420.3 and m/z 340.2 → 116.0 were monitored for linagliptin and its internal standard. Analyst® 1.7.3 software was used for sample acquisition, data processing and quantitation. 20 µL of plasma sample was added to 2 µL of internal standard and quenched with 60 µL of acetonitrile, vortexed for 30 sec and centrifuged at 10000 rpm for about 10 ºC for 10 min. The supernatant was transferred and injected into LC-MS/MS system. The method was linear over the range of 0.5 – 200 ng/mL with correlation coefficient greater than 0.996.

2.9 Statistical analysis

The data analysis was performed by using Graph pad prism software version - 8.4.2. All the values were expressed as Mean ± SD n = 8. Statistical difference between and within the

groups was analysed by using Two-way followed by Tukey's test. ($p<0.05$) was considered statistically significant.

3. RESULTS

3.1 Effect of linagliptin and also combination of both Linagliptin and piperine on Body Weight

In relation to Body weight, There is no significance between the Linagliptin, and also combination of both linagliptin with piperine groups when Compared with the Disease Control.

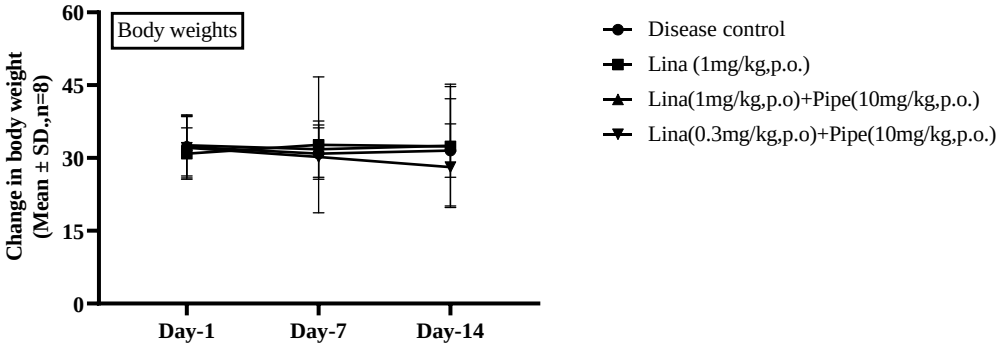


Figure 2: Effect of linagliptin and also combination of both linagliptin and piperine on Body weight. All the values were expressed as Mean ± SD.

3.2 Effect of Linagliptin and also combination of both Linagliptin and piperine on Blood Glucose Levels

The Results of blood glucose levels showed that Linagliptin alone was significantly decreased the hyperglycemic properties when compared to the Disease Control and also both the combination groups of linagliptin with piperine improved lowering of blood glucose as compared to the Disease control group. Fig.3; [$F(3,105) = 9.398$, ($p<0.05$), ($p<0.01$)].

However, There is no significance between the combination groups of linagliptin with piperine as compared to the Linagliptin alone.

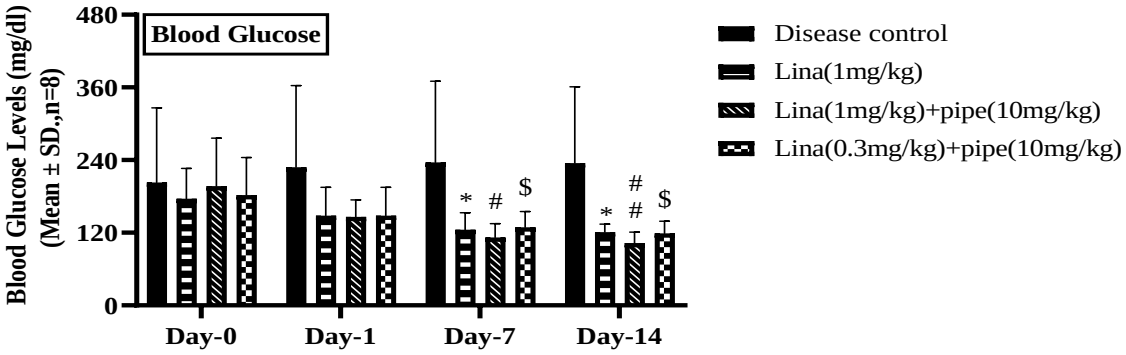


Figure 3: Effect of Linagliptin and also combination of both Linagliptin and piperine on Blood Glucose Levels. All the values were expressed as Mean ± SD, *P < 0.05, $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\$}$p<0.05 when compared with the Disease control group.

3.3 Effect of Linagliptin and also combination of both Linagliptin and piperine on Food Consumption

Regarding Food intake, the outcomes indicated that Disease group when compared with Linagliptin, and also combination of both linagliptin with piperine groups were significantly decreased. Fig.4;[F(3,77)=13.82, p<0.001]

Nevertheless, In Disease control group the food consumption was significantly increased on Day 7 and 14 when compared to Day 0. While In Linagliptin alone group was significantly Increased on Day 7 and 14 when compared to Day 0. Fig.4; [F2,77) =6.551, P<0.01]

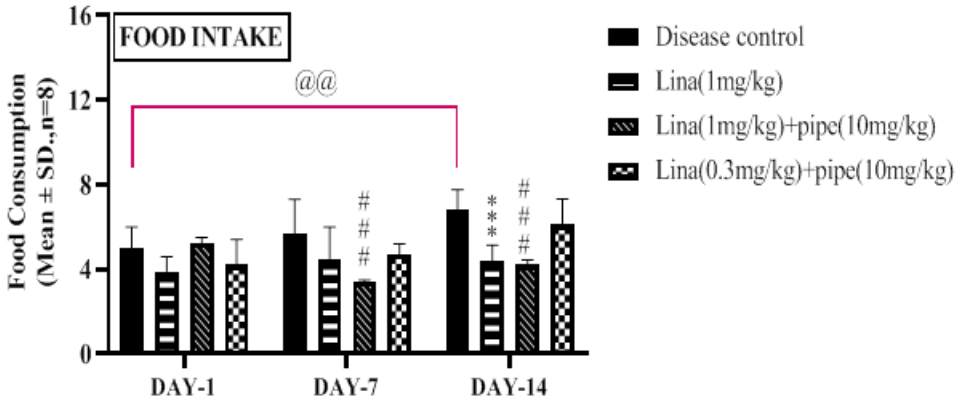


Figure 4: Effect of Linagliptin and also combination of both Linagliptin and piperine on Food consumption. All the values were expressed as Mean ± SD,*p<0.001$^{\#\#\#}$p<0.001, when compared with the Disease control group.$^{@@}$p<0.01when compared to the Day 14 on Disease Control.**

3.4 Effect of piperine on bioavailability of Linagliptin on day 8 The plasma concentration versus time profile of linagliptin in that the coadministration of piperine with linagliptin resulted in a significant increase in Cmax of linagliptin in mice plasma compared to linagliptin alone on day 8.

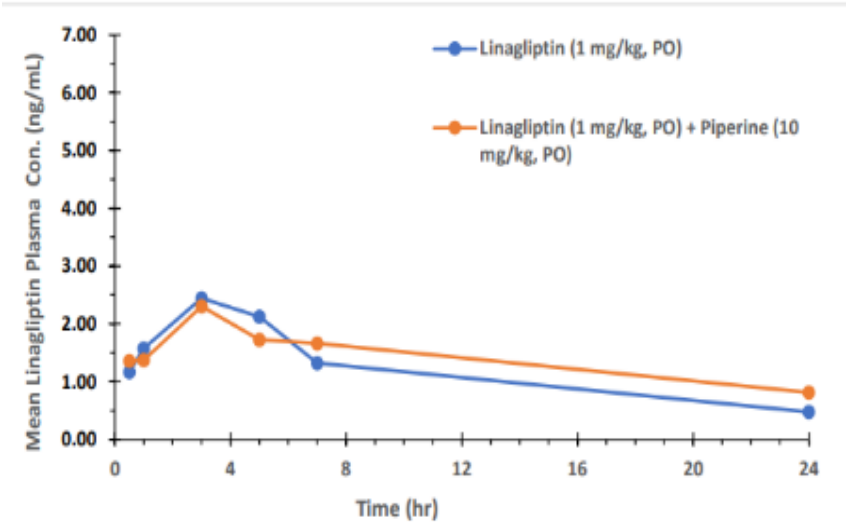


Figure 5: Effect of piperine on bioavailability of linagliptin on day 8.All the values were expressed as Mean ± SD.

3.5 Effect of piperine on bioavailability of Linagliptin on day 22

The plasma concentration versus time profile of linagliptin in that the coadministration of piperine with linagliptin resulted in a significant increase in the Cmax of linagliptin in mice plasma compared to linagliptin alone on day 22.

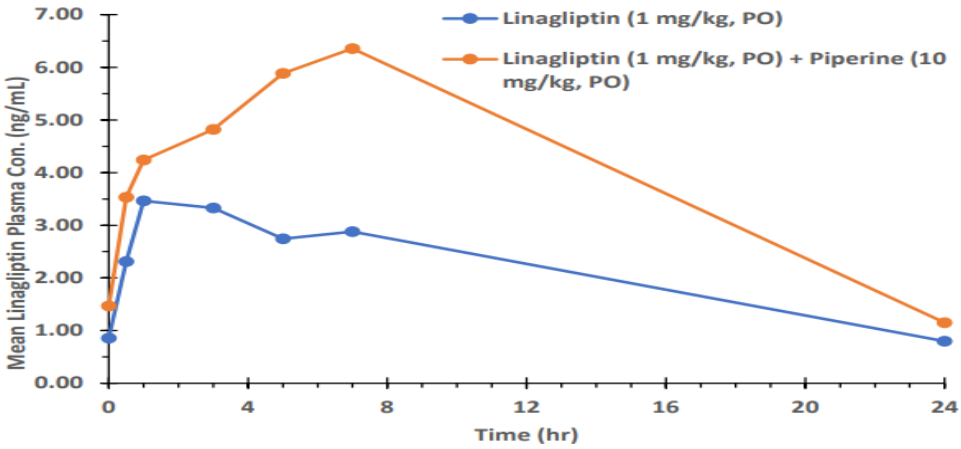


Figure 6: Effect of piperine on bioavailability of linagliptin on day22.All the values were expressed as Mean ± SD.

3.6 Pharmacokinetic parameters of Linagliptin alone and in combination with Piperine

In this study, we examined the pharmacokinetic (PK) parameters of linagliptin, both in its singular administration and when co-administered with piperine. The findings revealed a substantial increase in the maximum plasma concentration (Cmax) and the area under the curve (AUC) of linagliptin when it was combined with piperine, showcasing a pronounced escalation in bioavailability and systemic exposure as compared to linagliptin administered alone.

Table-3 Pharmacokinetic parameters of Linagliptin alone and in combination with piperine. (Values expressed as ng/ml are Mean ± SD).

In-vivo pharmacokinetic parameters of linagliptin following a single (Day 1) and repeated (14 days) linagliptin with and without piperine oral administration in in Alloxan induced diabetic CD1 mice.				
Treatment	**linagliptin (1 mg/kg, PO)**		**linagliptin (1 mg/kg, PO) + Piperine (10 mg/kg, PO)**	
PK Parameters	**Day 1**	**Day 14**	**Day 1**	**Day 14**
T_{max} (hr)	3.0	1.0 - 7.0	3.0	5.0 - 7.0
C_{max} (ng/mL)	2.4 ± 0.4	4.5 ± 0.7	2.3 ± 0.2	7.0 ± 2.8
AUClast (ng*hr/mL)	28.3 ± 4.2	52.0 ± 15.5	33.2* ± 4.0	99.0* ± 46.7
Note: Values are mean ± SD, n=3 animals/group.				

DISCUSSION

The current work adds to our understanding of the possible alternative therapy for Diabetes using rodent models. The goal of the current study was to assess the effects of piperine (10 mg/kg) and linagliptin [therapeutic(1mg/kg) and sub-therapuetic(0.3mg/kg)] administered together on alloxan monohydrate-induced diabetes in animals. In this work, we discovered that piperine has a bio-improving impact against alloxan monohydrate-induced Diabetes, which specifically kills beta cells—the insulin-producing cells in the pancreas—when given to mice, Through the GLUT2 glucose transporter, alloxan preferentially accumulates in pancreatic beta cells. Alloxan produces reactive oxygen species (ROS) as it enters the beta cells through a cyclic redox interaction with its reduction product, dialuric acid. Superoxide radicals, hydrogen peroxide (H_2O_2), and hydroxyl radicals are some of these ROS. Due to their weak antioxidant defense, beta cells are particularly vulnerable to hydroxyl radicals. Alloxan's cytotoxic effect is primarily mediated by the production of ROS, which results in the selective necrosis of beta cells.[125–127]

This study found that the active ingredient in black and long pepper, piperine, inhibits both human P-glycoprotein and CYP3A4. Linagliptin has the substrate P-glycoprotein (P-gp).

According to certain investigations, the bioenhancer piperine has been demonstrated to inhibit p-gp.[128,129] By inhibiting P-gp, piperine may increase the bioavailability of linagliptin when taken orally. This method can be applied to overcome the low oral bioavailability of linagliptin and enhance its therapeutic effect.[130–133]

According to previous research, following injection with alloxan monohydrate, animal body weights are reduced. It indicates high blood sugar levels. Alloxan is known to selectively kill insulin-producing cells in the pancreas, resulting in insulin shortage and subsequent hyperglycemia, which may account for the reduced body weight. As a result of the increased breakdown of fat and muscle tissue, this disruption in glucose metabolism may cause weight loss.[134–143]

A lack of insulin may cause a person to eat less, have less of an appetite, and lose weight. The pancreas secretes the hormone insulin, which is essential for controlling blood sugar levels and promoting the uptake of glucose by cells. Cells become starved for glucose when the body lacks enough insulin, which prevents glucose from entering cells efficiently. This may lead to a rise in hunger, a fall in satiety, and weight loss.[144]

However, there was no significant difference in body weight between the treatment group and disease control animals in this investigation. The lack of a noticeable change in body weight between the alloxan-induced animals and the treatment group may have been caused by the protein-rich meal given to the animals in the treatment group and also the disease control group.[145–150]

According to numerous studies, piperine significantly enhances the bioavailability of a variety of medication classes.[151] Following are a few generalized explanations for this phenomenon that have been demonstrated or advanced:

Improved absorption

Many medications have reduced gastrointestinal absorption, which results in low oral bioavailability. There are several proposed methods for how piperine increases the absorption of different medicines.[152] There Could be:

1) The increased solubility – By enhancing bile acid secretion or synthesis and inhibiting bile acid metabolism, piperine improves the formation of the micelle, which is necessary for

the absorption of lipids and lipid soluble medicines. The micelle is formed with the assistance of bile acid.

2) The expansion of blood supply-According to a study by Annamalai et al.[153] trikatu improves gastrointestinal blood flow, which increases medication absorption from the digestive system.

3) Altering epithelial cells to make them more permeable- Along with stimulating gamma-glutamyltranspeptidase activity and enhancing amino acid uptake by epithelial cells, piperine interacts with intestinal epithelial cells to improve medication absorption by the GI epithelium.[154] Piperine has also been proposed to improve brush border membrane fluidity and microvilli length.[155]

Slower metabolism

It has been discovered that piperine has a general inhibitory effect on xenobiotic and drug metabolism. It seems to inhibit several cytochrome P450 isoforms as well as hepatic arylhydrocarbon hydroxylase, UDP-glucuronyltransferase, and other related enzymes. By blocking the enzyme UDP-glucose dehydrogenase, it prevents the metabolic process of glucuronidation in isolated intestinal cells.[156,157] Piperine has also been shown to block a variety of mixed function oxygenases.[158] Piperine may also be a selective inhibitor of cytochrome P450 enzyme isoforms such as CYP1A1, CYP1A2, CYP2C8, CYP2D6, and CYP3A4.[159]

Prevention of solubilizer adherence: Substances are inhibited from entering cells when they are chemically coupled to a highly water - soluble molecule. Solubilizer attachment is the word for this. The chemicals attached to glucuronic acid, a key solubilizer, are eliminated in the urine or the small intestine. Piperine has been shown to block glucuronic acid, allowing for greater drug absorption into the cell.[160]

Drug efflux from the site of action is minimized: According to Bharadwaj et al.[160] piperine enhances drug retention at the active site by blocking human p-glycoprotein, a key efflux pump.[162]

According to earlier studies, metformin is a great example of piperine's bio-enhancer action. The CYP 450 family of enzymes (CYP2C11, CYP2D1) metabolize metformin, which has a rather low oral bioavailability of 40–60%.[163] As a result, piperine may significantly increase both the drug's bioavailability and its therapeutic efficacy. The mechanism of action of

metformin includes enhancing peripheral glucose utilization and enhancing blood glucose absorption. Therefore, piperine itself may have some additive effects at this lower dose, which would further boost the therapeutic impact. Similar to the commercially marketed combination of piperine with rifampicin, this combination would enable patients to take less metformin, decreasing both the drug's side effects and the expense of their treatment.[164]

The results of the current study showed that mice given alloxan had lower blood glucose levels and food intake. By administering Linagliptin and/or its combination with piperine as a treatment, the primary mechanism is the activity of piperine as a bioenhancer. Additionally, linagliptin, which has a very low oral bioavailability of 30%.[165] is metabolized by the CYP450 family of enzyme(CYP3A4), aldo-keto reductases, and carbonyl reductases. Piperine may thereby greatly improve the bioavailability of the medicine as well as its therapeutic effectiveness. The mean plasma concentration of linagliptin alone and in combination of both linagliptin with piperine will shows the significantly reduced the blood glucose levels by inhibiting the CYP3A4 and p-gp substrate[53,54] The incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are degraded by the enzyme DPP-4, which is one of the ways that linagliptin acts. When blood glucose levels are normal or raised, both GLP-1 and GIP cause pancreatic beta cells to produce and secrete more insulin[166,167] Therefore, at this lower dose, piperine itself may have some additive effects that would enhance the therapeutic benefit.

Finally, we showed that linagliptin and piperine coadministration revealed significant improvement in Blood glucose levels and food consumption, respectively, in the administration of Alloxan monohydrate induced diabetes model. Additionally, this study demonstrated that piperine has the potential to be employed as a bioenhancer in the treatment of diabetes when paired with linagliptin. Due to its anti-oxidant and anti-obesity qualities, piperine can also offer additional benefits in a condition like diabetes.

CONCLUSION

The study's findings make it abundantly clear that piperine has the potential to function as a bioenhancer when combined with linagliptin. As a result, it can be used to treat diabetes in a way that reduces the dosage of linagliptin, its side effects, and the cost of treatment. Due to its anti-oxidant and anti-obesity qualities, piperine can also offer additional benefits for a condition like diabetes. However, detailed bioavailability studies with piperine are required for the other oral anti-diabetic medications as well.

Author contributions

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Conflict of interest

Authors declared no conflict of interest.

Ethical approval

The animal experimentation protocol of this study was approved by the IAEC (Approval number: **(PD-BRC-23-001)**

Data availability

All data generated or analysed during this study was included in this article. The datasets used and/or analysed during the current study are available from the corresponding authors on reasonable requests.

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