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IMPROVED BIOAVAILABILITY OF LINAGLIPTIN BY RESVERATROL IN RATS-INVOLVEMENT OF PERMEABILITY GLYCOPROTEIN (P-gp) INHIBITION

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Keywords:

Resveratrol, Linagliptine bioavailability, Diabetes, Single-pass intestinal perfusion, P-gp inhibition

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ABSTRACT: The maintenance of an appropriate serum concentration of a drug is important to ensure therapeutic efficacy. Functional alterations of drug transporters may influence the serum concentration of drugs through changes in its pharmacokinetics and pharmacodynamics (PK/PD). In this study, we have focused on the influence of functional alterations in the intestinal P-glycoprotein (P-gp) on the PK of linagliptin (LIN) by the oral route under normal and diabetic conditions before and after pretreatment with resveratrol (RSV) and verapamil (VER) as standard. The interaction between LIN and RSV has been studied using *in-vitro* noneverted sac study, *in-situ* Single-Pass Intestinal Perfusion (SPIP) study, and *in-vivo* oral bioavailability study in rats. Pretreatment with RSV significantly enhanced the intestinal transport, apparent permeability, and effective permeability of LIN in normal and diabetic rats indicating the active role of P-gp in LIN absorption. Pretreatment with RSV significantly altered the C_{max} and AUC of LIN in both normal and diabetic rats. But compared to normal rats, diabetic rats show a significant decrease in intestinal transport, and increased oral bioavailability of LIN indicates that the expression of P-gp is increased in diabetic conditions. In conclusion, the oral bioavailability of LIN is improved with RSV, and this combination is beneficial for the better control of diabetes, and further studies are needed to confirm the results in patients.

INTRODUCTION: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high blood sugar, insulin resistance, and a relative lack of insulin. DM is mainly associated with the develop-ment and progression of pathological changes in various organ systems.

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Several reports have shown that diabetes may also alter the pharmacokinetic properties of many drugs [1-3,] and these changes are generally associated with changes in functional proteins, including drug metabolic enzymes and efflux transporters, which participate in the absorption, distribution, and elimination of drugs.

Currently, most of the drugs used in clinical practice are administered via the oral route, and intestinal P-glycoprotein (P-gp) plays a critical role in the uptake and absorption of its substrate drugs and acts as the first barrier for drugs. Furthermore, previous studies have demonstrated that P-gp

expression and function can be affected not only by inherited factors, such as genetic polymorphisms, but also environmental factors, such as dietary habits, medicines or several pathophysiological conditions including epilepsy, cancer, seizure, chronic renal failure, nonalcoholic fatty liver disease and diabetes [4-7]. Among these diseases, the prevalence of diabetes was estimated to be as much as 9.2-9.8% of the adult population worldwide in 2011, and this prevalence is expected to increase over the next 20 years [8, 9].

More interestingly, there are several reports focusing on changes in the expression and functional activity of P-gp in the brain, liver, kidney, and intestine, especially under diabetic conditions. A large number of anti-diabetic drugs have been found to be substrates for P-gp. It has been proposed that P-gp expressed in various tissues may have the potential to alter the distribution, metabolism and excretion processes of the anti-diabetic drugs under diabetic conditions. In contrast, because anti-diabetic drugs are generally used in the oral form, it is necessary to consider changes in the expression of intestinal P-gp, which plays a critical role in the absorption process of its substrate drugs administered *via* the oral route. However, there have been only a few reports that have focused on changes in intestinal P-gp and the influence of these changes on the pharmacokinetics of anti-diabetic drugs under diabetic conditions. Therefore, we have focused on the activity of intestinal P-gp under diabetic conditions. Linagliptin (LIN) is a selective, competitive dipeptidyl peptidase-4 (DPP-4) inhibitor, indicated for the treatment of type 2 diabetes. It follows non-linear pharmacokinetics and has a largely non-renal excretion route. It has been hypothesized that P-gp mediated intestinal drug efflux transport could influence LIN bioavailability and might contribute to its elimination [10].

In this study, we focused on the changes in the expression of P-gp in the intestine under diabetic conditions and the possible influence of diabetic conditions on the pharmacokinetics of linagliptin, which is a known substrate of P-gp. Resveratrol (RSV), a naturally occurring phytoalexin present in grapes, peanuts, and berries, is regularly consumed in the human diet [11]. RSV inhibits events associated with tumor initiation, promotion, and progression and also has potential cardioprotective effects. RSV has been reported to show an inhibitory effect on P-gp [12]. It has been reported to reverse the multidrug resistance in KBv200 cells by inhibiting the multidrug-resistant gene expression [13]. The objective of the present study was to investigate the influence of resveratrol pretreatment on intestinal transport and oral pharmacokinetics of linagliptin in normal and diabetic rats.

MATERIALS AND METHODS:

Chemicals and Animals: LIN was purchased from Aurobindo Pharma Ltd (Hyderabad, India). Resveratrol was obtained from Ambe Phytoextracts Ltd (New Delhi, India). Verapamil was procured from Lupin Labs (Pune, India). Phenol red was purchased from Hi-Media (Mumbai, India). Methanol HPLC, Acetonitrile HPLC, and DMSO have been purchased from E. Merck (India) Ltd Mumbai. Male Wistar rats weighing about 240–260 g were purchased from Mahaveera Enterprises, Ghatkesar road, Hyderabad, India. Rats housed in cages were kept under controlled temperature (20–22 ºC) and 12 h day-night cycle. Animals were used for studies after 1-week acclimatization with free access to water and food. The animal study protocol was reviewed and approved by the Institutional Animal Ethical Committee of Kakatiya University (Vidhyaranyapuri, Warangal, IAEC, TS, and India). All the animals were grouped and treated with the following regimens.

The rats were divided into six groups (3 groups-normal rats and 3 groups- diabetic rats, n=10. From all six groups, n=5 have been utilized for non-everted sac study, and another n=5 have been utilized for in situ single-pass intestinal perfusion (SPIP) study simultaneously. All the rats were orally treated with 0.25% w/v Sodium carboxy methylcellulose (Sod.CMC) as a vehicle. Diabetes was induced with single IP injections of STZ (45 mg/kg) in freshly prepared citrate buffer (0.1 M, pH 4.5,) [14].

Normal Rats:

Group-I (Control): Pre-treated orally with Sod. CMC for 7 days.

Group-II (RSV treated): Treated with RSV (50 mg/kg/oral) suspension Sod. CMC for 6 days and also on 7th day 1 h prior to the study.

Group-III (VER Treated): VER (10mg/kg) for 6 days and also on 7th day 1 h prior to the study.

Diabetic Rats:

Group-IV (Control): Pretreated orally with Sod. CMC for 7 days.

Group-V (RSV Treated): Treated with RSV (50 mg/kg/oral) suspension Sod.CMC for 6 days and also on 7th day 1 h prior to the study.

Group-VI (VER Treated): VER (10 mg/kg) for 6 days and also on 7th day 1 h prior to the study.

Non-everted Sac (Normal sac) Study: All the groups were subjected to isolate the ileum sacs and filled with LIN 1000 µg/mL according to the prescribed procedure15. Briefly, in rats with free access to water before the experiment, all the rats were exsanguinated under anesthesia using thiopental sodium (50 mg/kg/ip); the ileum (10 cm length) separated and was flushed with 50 mL of ice-cold saline. The ileum sac preparations were filled with LIN 1000 µg/mL, which was dissolved in a fluid containing isotonic Dulbecco's Phosphate Buffer Saline (D-PBS), 25 mM glucose, and 0.5% of DMSO. The LIN solution (1 mL) was filled into the non-everted ileum sac (mucosal side), and both ends of the sac were ligated tightly.

The sac containing LIN solution was immersed into beaker containing 40 mL of D-PBS, 25 mM glucose, and 0.5% of DMSO. The medium was pre-warmed at 37 OC and pre-oxygenated with 5% CO2/ 95% O_2 for 20 minutes. Under controlled bubbling with a CO_2/O_2 mixture gas, the transport of the LIN from mucosal to serosal surfaces across the intestine was measured by sampling the serosal medium periodically for 120 min. The samples of 1 mL were collected at predetermined time intervals from the serosal medium and replenished with fresh buffer. The drug transported was measured using high-performance liquid chromatography (HPLC) [16].

Apparent Permeability Coefficient (Papp): The apparent permeability coefficient for linagliptin was calculated from the equation given below.

$$Papp = dQ/dt \times 1 / AC0$$

Where, dQ/dt is the rate of drug transport (linagliptin) from mucosal to serosal medium. A is the surface area of the intestinal sac used for the study. C0 is the initial concentration of drug present in the intestinal sac [17].

***In-situ* Single-pass Intestinal Perfusion (SPIP) Study:** The *in-situ* single-pass intestinal perfusion (SPIP) study was performed according to the previously reported methods [18]. Briefly, rats were anesthetized using thiopental sodium (50 mg/kg, ip) and they were placed on a warm pad to maintain normal body temperature. A small midline incision of 2-3 cm was made on the abdomen portion of rats, and an ileum segment of approximately 8-12 cm was isolated using the ileo-caecal junction as a distal marker.

Semicircular incisions were made at each end of the ileum, and the lumen was rinsed with normal saline (37 ºC), and the; both ends were cannulated with polyethylene tubing and ligated by using silk suture. Then, blank perfusion buffer (phosphate buffer saline, pH 7.2) was first perfused for 5 min at a flow rate of 1 mL/min by using a Syringe pump (NE- 1600, New Era Syringe Pumps, Inc. NY, USA), followed by perfusion of phosphate buffer saline (pH 7.2) containing LIN 150 µg/mL, and phenol red 0.2 mg/mL at a constant flow rate of 0.2 mL/min for a period of 90 min and perfusate was collected at every 10 min interval. After completion of cannulation, the ileum segment was covered with isotonic saline-wet gauze (37 ºC). At the end of perfusion, the length of the ileum segment was measured following the last sample collection. The samples were collected from control and pretreatment groups at predetermined time intervals and stored at -40 ºC until analysis. Linagliptin concentrations in perfusion samples were analyzed by HPLC.

Phenol Red Water flux Correction: The corrected outlet concentration (Cout (corr)) for linagliptin was calculated from the following equation [19].

Cout (corr)) = Cout × Concentrationof phenol red in (CPRin) / Concentrationof phenol red out CPRout

Where Cout (corr) is corrected outlet concentration of the linagliptin, Cout is the outlet concentration of the drug, whereas CPRin and CPRout are the concentration of phenol red entering and exiting the rat intestinal segment, respectively.

Effective Permeability Coefficient (Peff): The effective permeability coefficient of linagliptin was calculated from the following equation.

$$Peff = -Q \times in (Cout (corr)) / Cin / 2\pi rL$$

Where, Q is perfusion flow rate , C out(corr) is corrected outlet concentration of the drug, Cin is inlet drug concentration, r is radius of the rat small intestine, and L is the length of the perfused intestinal segment.

Peff was estimated from the steady-state concentration of compounds which is considered to be attained when the concentration of phenol red in the perfusate samples is stable. Generally, the steady-state was reached at 30-40 min after the beginning of the experiment.

***In-vivo* Bioavailability Study in Male Wister Rats:** In another set of study the rats n=5 were grouped according to the following protocol to perform PK studies

Normal Rats:
Group-I (Control): Pre-treated orally with Sod.CMC for 7 days with LIN 10 mg/kg/oral.

Group-II (RSV Treated): Treated with RSV (50 mg/kg/oral) suspension Sod.CMC for 7 days in combination with LIN 10 mg/kg/oral.

Group-III (VER Treated): VER (10 mg/kg) for 7 days in combination with LIN 10 mg/kg/oral.

Diabetic Rats:
Group-I (Control): Pre-treated orally with Sod. CMC for 7 days with LIN 10 mg/kg/oral.

Group-II (RSV Treated): Treated with RSV (50 mg/kg/oral) suspension Sod. CMC for 7 days in combination with LIN 10 mg/kg/oral.

Group-III (VER Treated): VER (10 mg/kg) for 7 days in combination with LIN 10 mg/kg/oral.

The animal study protocol was reviewed and approved by the Institutional Animal Ethical Committee, University College of Pharmaceutical Sciences, Kakatiya University, India. Male Wister rats weighing 180-250 g were selected for the study. The bioavailability of LIN after pretreatment with RSV was compared with an oral dispersion LIN. The rats were allowed free access to food and water until the night prior to dosing and were fasted for 10 h. Blood samples (0.5 mL) from retro-orbital vein were collected at preset intervals of 0, 0.5, 1, 2, 3, 6, 12, 24 and 36 h respectively. All blood samples were allowed to clot and centrifuged for 10 min at 4000 rpm. The serum was separated and transferred into clean microcentrifuge tubes and stored at -20 °C until HPLC analysis. The concentration of LIN in the samples was estimated using HPLC [20].

Serum and Perfusion Samples Analysis: LIN in the serum and perfusion samples was estimated by reverse-phase high-pressure liquid chromatography method.

Analysis of Intestinal sac and Perfusion Samples: A Shimadzu HPLC system equipped with a LC- 20AT pump and SPD 20 AVP UV visible detector and RP C18 column (250 mm×4.6 mm ID, particle size 5 µm, (Phenomenex, Kondapur, Hyderabad, India) was used for the HPLC analysis of serum and perfusion samples. The mobile phase used was 50 mM potassium dihydrogen orthophosphate (pH- 4.6): acetonitrile: 70:30, and the elution was monitored at 240 nm with a flow rate of 0.6 mL/min. The retention time of linagliptin was 6.2 min.

Analysis of Serum Samples: The mobile phase was consisted of 0.1% formic acid (pH 4.1) and methanol at the ratio of 75:25 v/v% and delivered at a flow rate of 1.0 ml min−1. The UV detector was set at 240 nm. The retention time of linagliptin was found to be 5.8 min. Metformin was used as an internal standard, and its retention time was found to be 7 min. Partial validation of the bioanalytical method has been done to measure the accuracy, precision, limit of detection, and quantification of the analyte.

Sample Preparation: To 100 µL serum, 100 µL of internal standard (50 ng/mL) and 100 µL of 1M/L sodium hydroxide were added and vortexed for 10 s. To this mixture, 3 mL of ethylacetate was added, then samples were vortex-mixed for 2 min and centrifuged at 2,500 × g for 15 min. The organic layer was carefully separated and transferred into another clean tube, and evaporated to dryness. The residue was reconstituted by adding 75 µL of the

mobile phase, vortexed for 10 s filtered through 0.2 µm syringe filter, and then a volume of 20 µL was injected to the HPLC system for analysis.

Statistical Analysis: The pharmacokinetic parameters were analyzed by using Phoenix Win Nonlin software version 6.2 (Certara, Pharsight Corporation, L.P, USA). Data are presented as means ± SD. Prism 5.0 software (GraphPad, LaJolla, CA, USA) was used for data analysis. Statistical analysis was performed using One-way ANOVA and followed by Dunnett's multiple comparison tests. Differences were considered statistically significant at $p<0.05$.

RESULTS:

***In-vitro* Noneverted sac Study:** The results of intestinal transport (normal rats) showed that resveratrol pretreatment for 7 days resulted in a significant increase in the mean cumulative concentration of LIN from 2.53 ± 0.1 to 4.12 ± 0.06 µg/mL, whereas for the VER (standard inhibitor) treated group the mean cumulative concentration of LIN was observed to be 4.77 ± 0.247 µg/mL. The transport of LIN was increased 1.6 and 1.9 times after treatment with RSV and VER respectively, compared to control in the ileum region of the normal rats **Fig. 1**. A statistically significant ($p<0.05$) difference was observed in both cases. The results of intestinal transport (diabetic rats) showed that RSV pretreatment for 7 days resulted in a significant increase in the mean cumulative concentration of LIN from 2.03 ± 0.110 to 4.74 ± 0.133 µg/mL, whereas for the VER treated group, the mean cumulative concentration of LIN was observed to be 5.85 ± 0.475 µg/mL.

The transport of LIN was increased 2.3 and 2.9 times after pretreatment with RSV and VER, respectively, compared to their respective control in the ileum region of the diabetic rats **Fig. 2**.

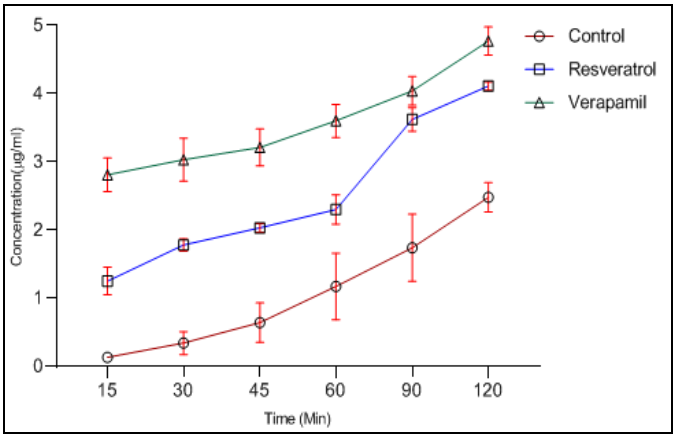

FIG. 1: CUMULATIVE TRANSPORT OF LINAGLIPTIN (1000 µG/ML) IN ILEAL NON-EVERTED SACS OF NORMAL RATS (N=5)

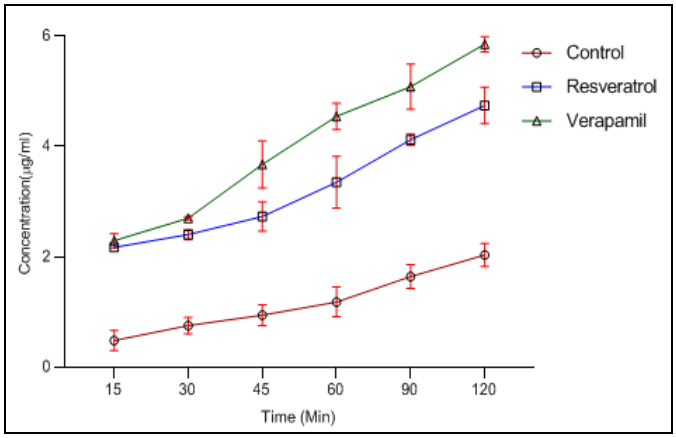

FIG. 2: CUMULATIVE TRANSPORT OF LINAGLIPTIN (1000 µG/ML) IN ILEAL NON-EVERTED SACS OF DIABETIC RATS (N=5)

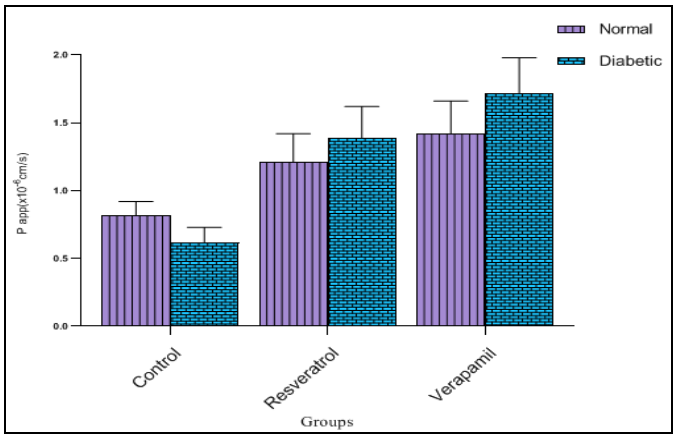

FIG. 3: EFFECT OF RESVERATROL ON APPARENT PERMEABILITY OF LINAGLIPTIN IN NORMAL AND DIABETIC RATS, N=5

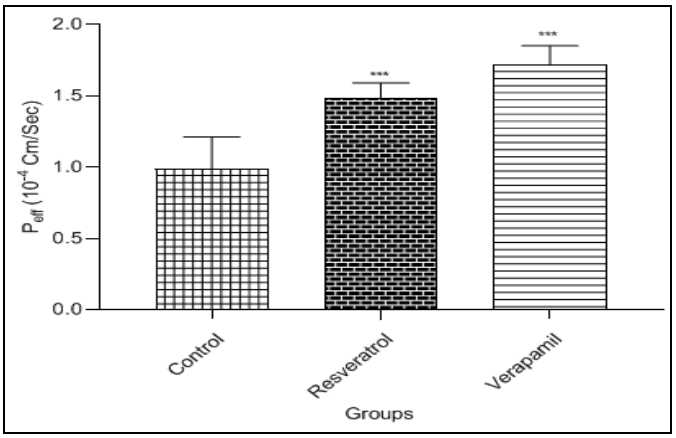

FIG. 4: EFFECT OF RESVERATROL ON EFFECTIVE PERMEABILITY OF LINAGLIPTIN (150 MCG/ML) IN NORMAL RATS

A statistically significant ($p<0.05$) difference was observed in both cases. LIN intestinal apparent permeability (Papp) was determined in the rat ileum segment using a non-everted sac study. The RSV pretreatment for 7 days significantly enhanced the apparent permeability of LIN **Fig. 3**. The apparent permeability of LIN was increased 1.6 and 1.9 times after pretreatment with RSV and VER, respectively, compared to their respective control in normal rats. The apparent permeability of LIN was increased 2.3 and 3.0 times after pretreatment with RSV and VER, respectively, compared to their respective control in diabetic rats **Fig. 3**. A statistically significant difference was observed in both cases ($p<0.05$, **Fig. 3.**

In-situ Single-Pass Intestinal Perfusion (SPIP) Study: LIN intestinal effective permeability (Peff) was determined in the rat ileum segment using single-pass intestinal perfusion technique. Effective permeability values were calculated from the steady-state concentrations of compounds in the perfusate collected from the outlet. Pretreatment with resveratrol for 7 days (RSV treated group) and concomitant administration with standard inhibitor VER resulted in a significant ($p < 0.05$) increase in effective permeability of LIN in both normal and diabetic rats. The increase in effective permeability of LIN in the ileum was found to be 1.5 and 2.0 folds in pretreated and standard inhibitor group, respectively as compared with that of the control group in normal rats **Fig. 4** and **5**.

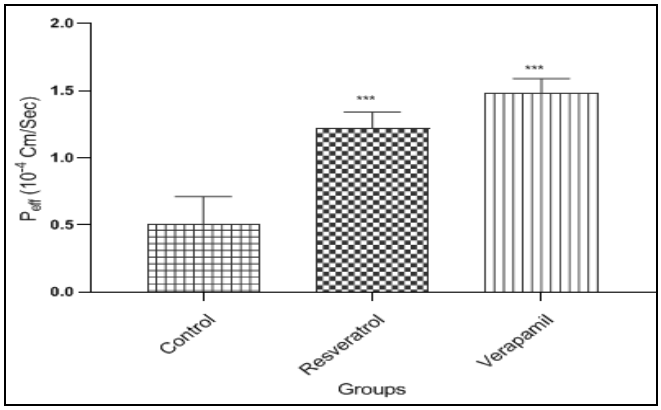

FIG. 5: EFFECT OF RESVERATROL ON EFFECTIVE PERMEABILITY OF LINAGLIPTIN (150 MCG/ML) IN DIABETIC RATS. Data represent Mean ± SD, n=5. * Significant difference ($p<0.05$) compared to control group.

The increase in effective permeability of LIN in the ileum was found to be 2.6 and 2, 8 -fold in pretreated and standard inhibitor groups respectively as compared with that of the control group in diabetic rats. A statistically significant difference was observed in both cases ($p<0.05$).

***In-vivo* Study:** All the rats tolerated the treatments well, and there were no cases of severe adverse effects during the study period. The serum concentration-time profile of LIN after oral administration in control, RSV (50 mg/kg) pretreated, and VER (10 mg/kg) treated groups were characterized and are depicted in **Fig. 6** and **Fig. 7**. The mean pharmacokinetic parameters of LIN are summarized in **Table 1** and **Table 2**.

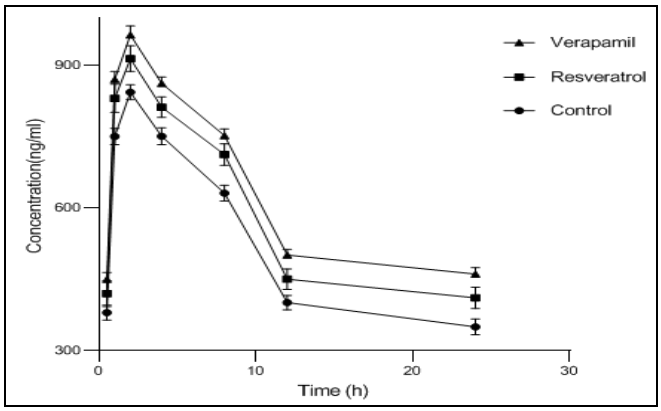

FIG. 6: SERUM DRUG CONCENTRATION- TIME PLOTS OF LINAGLIPTIN (10 mg/kg) IN NORMAL RATS. Data are represented as Mean ± SD (n=5)

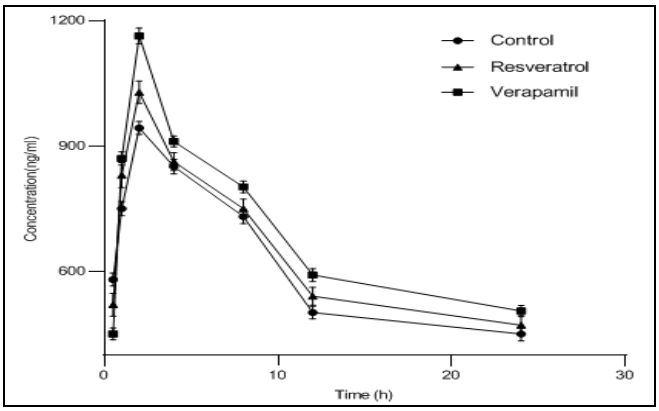

FIG. 7: SERUM DRUG CONCENTRATION TIME PLOTS OF LINAGLIPTIN (10 mg/kg) IN DIABETIC RATS. Data are represented as Mean ± SD (n=5).

LIN oral pharmacokinetics was found to be significantly ($p<0.05$) altered with RSV pretreatment for 7 days compared to the control group.

The increases in Cmax, AUC 0–t, and AUC0-∞ of LIN were found to be 1.1, 1.2, and 1.3- fold respectively in the resveratrol pretreated group compared to that of the control group of normal

rats. The increases in Cmax, AUC 0–t, and AUC0-∞ of LIN were found to be 1.2, 1.2, and 1.3- fold respectively in the VER treated group compared to that of the control group of normal rats. The increases in C_{max}, AUC 0–t, and AUC0-∞ of LIN were found to be 1.2, 1.4, and 1.5- fold respectively in the resveratrol pretreated group compared to that of the control group of diabetic rats. The increases in C_{max}, AUC 0–t, and AUC0-∞ of LIN were found to be 1.2, 1.4, and 1.6- fold respectively, in the VER treated group compared to that of the control group of diabetic rats. There was a statistically significant difference observed in pharmacokinetic parameters, C_{max}, T_{max}, AUC0-∞, AUC 0-t, Vd, and clearance.

TABLE 1: PHARMACOKINETIC PARAMETERS OF LINAGLIPTIN (10 mg/kg) IN CONTROL, RESVERATROL AND VERAPAMIL TREATED GROUPS IN NORMAL RATS

Pk Parameters	Control	RSV	VER
C_{max} (ng/mL)	911.42 ± 10.63	1037.79 ± 19.95*	1040.30 ± 22.59*
T_{max} (hr)	1	1	1
K_{el} (h^{-1})	0.034 ± 0.001	0.03 ± 0.001	0.029 ± 0.0006
AUC $_{0-t}$ (ng/mL/hr)	19266.06 ± 434.1	24012.69 ± 318.85*	23808.45 ± 276.12*
AUC $_{0-\infty}$ (ng/mL/hr)	25774.97 ± 925.59	34307.89 ± 789.34*	34580.14 ± 899.76*
Clearance (mL/hr)	116.51 ± 4.19	87.48 ± 2.01*	86.80 ± 2.2*4
Vd (mL)	3401.21 ± 101.18	2914.11 ± 35.66*	2980.53 ± 12.52*

Data represents Mean ± SD, n=5. RSV= Resveratrol, VER = Verapamil * Significant difference ($p<0.05$) compared to control group.

TABLE 2: PHARMACOKINETIC PARAMETERS OF LINAGLIPTIN (10 mg/kg) IN CONTROL, RESVERATROL AND VERAPAMIL TREATED GROUPS IN DIABETIC RATS

Pk Parameters	Control	RSV	VER
C_{max} (ng/mL)	834.48 ± 11.61	1032.37 ± 1.82*	1020.28 ± 10.50*
T_{max} (hr)	1	1	1
K_{el} (h^{-1})	0.037 ± 0.0008	0.038 ± 0.0007	0.028 ± 0.0008
AUC $_{0-t}$ (ng/mL/hr)	16592.29 ± 222.3	23228.98 ± 155.33*	23722.54 ± 332.51*
AUC $_{0-\infty}$ (ng/mL/hr)	21483.54 ± 549.12	33125.88 ± 406.07*	34772.92 ± 813.80*
Clearance (mL/hr)	139.71 ± 3.55	94.85 ± 1.76*	86.31 ± 2.02*
Vd (mL)	3746.41 ± 23.26	3156.68 ± 17.25*	3010.32 ± 58.63*

Data represents Mean±SD, n=5. RSV= Resveratrol, VER= Verapamil * Significant difference ($p<0.05$) compared to control group.

DISCUSSION: With the great interest in herbal products as complementary and alternative medicines, much effort is currently being expanded toward identifying natural products of plant origins that modulate efflux transporters as well as metabolic enzymes. However, there is less information on the pharmacokinetic interactions between natural products and conventional medicines.

Therefore, more preclinical and clinical studies on the herbal drug interaction should be conducted to prevent potential adverse reactions or utilize those interactions for a therapeutic benefit. Therefore, the present study evaluated the effects of resveratrol, an antioxidant, on the bioavailability or pharmacokinetics of LIN in rats to examine a potential drug interaction between RSV and LIN via inhibition of P-gp. Drug transporters are classified as solute carrier (SLC) transporters and ATP-binding cassette (ABC) transporters. SLC transporters, including organic anion transporters (OAT) and organic cation transporters (OCT) will carry specific drug substrates through passive transport or co-transport. In contrast, ABC transporters, including P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-associated protein-2 (MRP-2) utilize energy from ATP hydrolysis to transport many structurally unrelated drugs. P-gp, the best-studied ABC transporter, is present not only in tumor cells but also in various normal tissues, such as brain, lungs, liver, kidneys, intestine, skin and muscle tissue [21, 22]. P-gp is considered as an important component of the blood-brain barrier (BBB), blood-placenta barrier, blood-testis barrier and other biological barriers *in-vivo* [23, 24]. P-gp is located on the apical membrane of the small and large intestine and regulates the absorption processes of various classes of drugs from the intestine to the systemic circulation by transporting them to the intestinal lumen [25].

Because P-gp plays an important role in tissues to pump out the large number of its substrates often used in the clinic, including anticancer drugs, antihypertensive drugs, antihyperlipidemic drugs, and others, many researchers have studied the influence of changes in expression and functional activity of P-gp on the pharmacological effects of its substrates [26]. Inhibition or induction of this transporter protein was implicated as a mechanism underlying certain herbal-drug interactions. In the clinical setting, familiarity with known P-gp inhibitors should increase awareness of potential adverse or desired effects when phytochemicals interact with P-gp substrates.

There is a variety of methods that have been developed to measure drug transport across the gastrointestinal tract. The methods include in situ single-pass intestinal perfusion through intestinal segments, *in-vitro* diffusion across tissues, and *in-vitro* permeation through cell monolayers or artificial membranes. The interaction between linagliptin and resveratrol has been studied using *in-vitro*, *in-situ*, and *in-vivo* models.

In the non-everted gut (or intestinal) sac model, a section of the intestine is removed from an anesthetized rat and flushed with ice-cold saline. The intestine is divided into 8-10 cm sacs which are filled with oxygenated buffer containing a drug, tied at each end, and placed in a container of a well-mixed oxygenated buffer. After a specified time period, the amount of drug in the buffer is measured, and Papp is calculated. The *in-vitro* noneverted rat gut sac model is the most direct method of identifying the absorption of the drug from a mucosal to serosal direction. A significantly lower permeability in the mucosal to serosal direction provides evidence that some form of efflux transporters such as P-gp is inhibiting the transport of test component [27]. The results from a non-everted sac study revealed that the transport of LIN across the rat intestine is very much affected by resveratrol. In the study, the mean ± SD cumulative concentration of LIN and apparent permeability were shown to increase after pretreatment with resveratrol for 7 days. This observation indicated the role of P-gp, an efflux pump, on linagliptin absorption. Inhibition of intestinal P-gp by zosuquidar increased the bioavailability of orally administered linagliptin, indicating that this transport system plays a role in limiting the uptake of LIN from the intestine [28]. RSV showed a significant improvement in the oral bioavailability of diltiazem by inhibiting P-gp mediated drug efflux in the rat intestine [28]. Further, in order to support the role of P-gp inhibition by resveratrol involved in intestinal transport of linagliptin, *in-situ* perfusion studies were performed at the ileum part of the rat intestine. Estimation of clearance pathways, presence of enzymes and transporters across the gastrointestinal mucosa were effectively studied by perfusion technique.

The *in-situ* perfusion model allows the measurement of drug transport in the intact intestine by the single-pass method. For the single-pass (open loop) perfusion technique, a section of intestine in an anesthetized rat is cannulated proximally and distally, rinsed with buffer solution, and then perfused with a drug solution. The amount of drug in the perfusate is measured at predetermined time intervals. Intestinal effective permeability (Peff) is calculated from the difference between solute concentration entering and leaving the cannulated region.

From this technique, we can also assess the actual extent of P-gp efflux that can be expected in vivo. In the present study, the effective permeability of linagliptin was significantly increased after pretreatment with resveratrol. Thus, the increase in linagliptin intestinal permeability in pretreated rats might be attributed to the P-gp inhibition by resveratrol. Phenol red, a non-absorbable marker, was used to provide information on the integrity of the intestinal membrane [29]. Furthermore, we have also studied the intestinal transport of linagliptin in normal and diabetic rats. Compared to normal rats, diabetic rats showed a significant difference in mean cumulative concentration, apparent per-meability and effective permeability of linagliptin indicate that P-gp expression is altered (increased) in diabetic conditions. The expression of intestinal P-glycoprotein significantly increased with the progression of diabetes which was inferred from the mRNA analysis of mdr1a and mdr1b genes in the ileum segment of rat intestine [30].

The results from the in vivo oral pharmacokinetic study showed a significant increase in AUC, $C_{max,}$

and volume of distribution. The higher plasma levels in the absorption phase of linagliptin may be due to inhibition of P-gp across the rat intestine. Further, the CL/F was significantly decreased while there was no significant change in T1/2, and Kel of linagliptin was observed in the pretreated group as compared to the control group. The lack of significant changes in T1/2 and Kel may indicate that resveratrol has negligible effects on hepatic elimination of linagliptin. Thus, pretreatment with resveratrol significantly enhanced the AUC and C_{max} of linagliptin, likely by the increased intestinal absorption of linagliptin via the inhibition of P-gp mediated drug efflux, which in this study has been substantiated with noneverted intestinal sac and in situ permeability data. Pretreatment with resveratrol significantly enhanced the AUC and C_{max} of linagliptin, which may be due to increased intestinal absorption of linagliptin via the inhibition of P-gp mediated drug efflux.

CONCLUSION: The results confirmed the possibility that the increased bioavailability of linagliptin in the presence of resveratrol might be associated with the inhibition of P-gp mediated drug efflux. Thus, there is potential pharma-cokinetic interaction between resveratrol and linagliptin has been observed. In the study, we have focused on alterations of intestinal P-gp under diabetic conditions. From the results, it became clear that resveratrol inhibits P-gp and improves the oral bioavailability of linagliptine. When LIN combined with RSV is beneficial and may reduce the dose of LIN, however, further studies are needed to confirm the results in patients.

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