MS.ID-IJPBS-6040
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2022) 12 (4): 238-247
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

Effect of Andrographolide on the Pharmacokinetics and Pharmacodynamics of Repaglinide in Diabetic Rats

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Received: 02 Jul 2022/ Accepted: 9 Aug 2022 / Published online: 1 Oct 2022
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Abstract

As aboriginal sources of medications, medicinal plants have been used since ancient times. *Andrographis paniculata* is one of the highly used potential medicinal plants in the world. This plant is traditionally used for the treatment of common colds, diarrhea, fever due to several infective causes, jaundice, as a health tonic for the liver and cardiovascular health, and as an antioxidant. It is also used to improve sexual dysfunctions and serve as a contraceptive. All parts of this plant are used to extract the active phytochemicals, but the compositions of phytoconstituents showed that repaglinide levels was increased in single dose and multidose treatment of repaglinide, may be due to metabolic inhibition of CYP3A4. It indicates that occurrence of drug interaction, which may be due to decreased metabolism of repaglinide. According to the results pharmacokinetics and pharmacodynamics of repaglinide are more pronounced in multi dose treatment groups of combination, it indicates the significance of long-term exposure of Andrographolide in diabetic condition, thus it may apply to diabetic patients under Repaglinide treatment. Hence, the combination has a beneficial effect in diabetic conditions, but special concern must be observed in diabetic patients in view of the side effects of Repaglinide.

Keywords

Andrographis paniculata, Cancer, Protein kinase, repaglinide.

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1.0. INTRODUCTION:

Medicinal plants have been used to treat human diseases since the beginning of human civilization. More than 80,000 plant species have been identified and used as medicinal plants around the world, according to estimates. More than 1300 plant species have been traditionally used in Malaysia, where knowledge is passed down from generation to generation. The indigenous medicinal plants and plant-derived drugs are the potential source of alternative medicine and are extensively used to treat various health ailments [Kavishankar. G *et al*., 2011]. Use of the medicinal plants is a core component at primary health care level due to availability, acceptability, compatibility, and affordability. Dependency on these medicinal plants varies from country to country. It is estimated that about 75–80% of people of developing countries and about 25% of people of developed countries depend either directly or indirectly on medicinal plants for the first line of treatment. Therefore, people are encouraging indigenous production and processing of these medicinal plants to use in different cultures and religion for the treatment of various diseases. Moreover, the importance and

uses of medicinal plants are also stated in different religious books. About 19 medicinal plants and 176 medicinal plants are mentioned in the Holy Qur'an and the Holy Bible, respectively [Urbi.Z *et al*., 2014 and Duke J.A *et al*., 2007].

Andrographis paniculata (AP) is an important medicinal plant and widely used around the world. It belongs to the family *Acanthaceae*. AP is used as a traditional herbal medicine in Bangladesh, China, Hong Kong, India, Pakistan, Philippines, Malaysia, Indonesia, and Thailand and is ethnobotanically used for the treatment of snake bite, bug bite, diabetes, dysentery, fever, and malaria [Akbar. S *et al*., 2011 and Kabir M. H. *et al*., 2014]. In the Unani and Ayurvedic medicines, AP is one of the most used medicinal plants. Commercial preparations of this plant's extract have recently been employed in some countries. To improve their efficacy, the preparations must yet be standardized. The most widely utilized section of AP is the aerial portion, and its extracts contain lactones, flavonoids, diterpenoids, and flavonoid glycosides. Whole plant leaves and roots are also used as a folklore remedy for different diseases in Asia and Europe [Jarukamjorn. K *et al*., 2008]. AP has been reported to have a broad range of pharmacological effects including anticancer antidiarrhea, antihepatitis, anti-HIV, antihyperglycemic, anti-inflammatory [Chiou W.F *et al*., 2000, Sheeja. K *et al*., 2006, Shen Y. C *et al*., 2000 and Shen Y. C *et al*., 2002] and antimicrobial, antimalarial, antioxidant, cardiovascular, cytotoxic, hepatoprotective, immunostimulatory, and sexual dysfunctions [Akbarsha M. A *et al*., 2000].

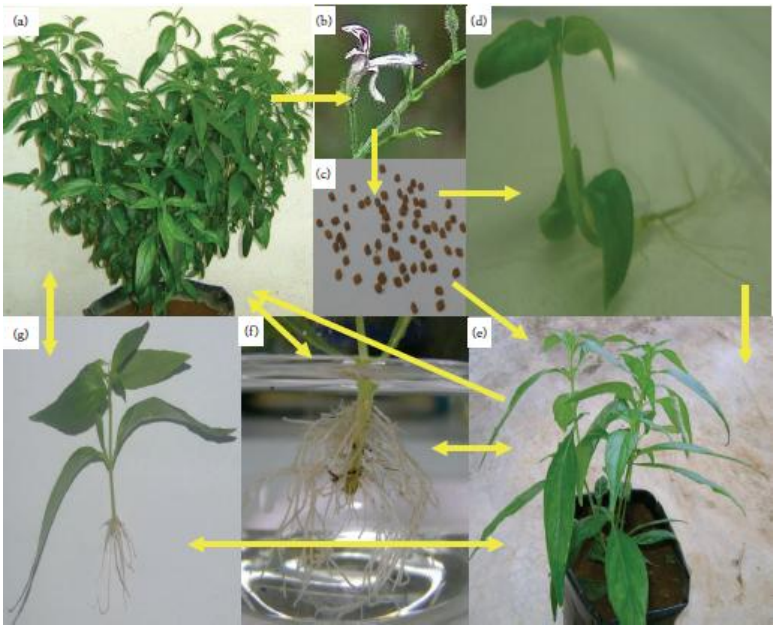

Figure 1: Morphology of Andrographis paniculata. (a)Mature A. paniculata in polybag stage, (b) flowering stage, (c) harvested seeds, (d) *in vitro* seedling, (e) young *A. paniculata* in polybag, (f) adventitious roots of *A. paniculata*, and (g) vegetative seedlings. Single direction of arrow indicates the developmental stages and both directions of arrow denote vegetative propagation of plant.

2.0. OBJECTIVE OF THE STUDY

To determine the effect of Andrographolide on the pharmacokinetics and pharmacodynamics of Repaglinide in diabetic rats.

Need of the work

Patients have many concerns when multiple medications are started, including prescribing errors, the cost of medications, and possible adverse effects. Significantly, 58% of patients worry that they will be given medications that have drug interactions that will adversely affect their health.

Interactions between herbal medicines and prescribed drugs can occur and may lead to serious clinical consequences.

Thus, there is need to consolidate the clinical and pharmacologic aspects of drug-herb interactions, to develop a compendium of information, to provide a measure of the risk of interactions, a description of the clinical consequences, and an assessment of the quality (i.e., validity) of evidence.

CYP inhibiting drugs increase the concentration of the drugs that are substrates for the specific CYP isoform and thus enhance the pharmacological and

toxicological effects of the substrate drugs. Repaglinide is a CYP3A4 substrate and Andrographolide is CYP3A4 strong inhibitor. Hence, there is a chance of influence of herbs on the pharmacokinetics and pharmacodynamics of repaglinide. Moreover, Andrographolide used for the treatment by the Diabetic patients, there is a chance for herb-drug interactions.

3.0. MATERIALS AND METHODS:

3.1.1. Drugs and Chemicals

Methanol HPLC grade (Merck, Mumbai) , Andrographolide (Yucca Enterprises), Repaglinide (Novartis, Hyderabad) , Gliclazide (Novartis, Hyderabad) , Streptozotocin (Sigma Aldrich, Bangalore) , Sodium citrate dehydrate , Citric acid , Diethyl ether.

3.1.2. EQUIPMENTS

HPLC (contains C18 column coated with 4micron particles), Biofuge (Heraeus instrument- Germany), Micropipettes (Tarsons), Microcentrifuge tubes (Tarsons), Heparinized capillaries.

Ultra sonicator (Solitec, Spincotech PVT LTD), Glucometer and Glucometer strips.

4.0. Pharmacological evaluation:

4.1. Study design

Male Wister rats, weighing between 180-250g, were procured from Jeeva agencies, Hyderabad. They were maintained under standard laboratory conditions at ambient temperature. They were fed with a standard pellet diet and water *ad libitum.* The food was withdrawn from the animal cages 12 hours before the experiment and during experiment.

The experimental protocol was ethically approved by Institutional Animal Ethical Committee (IAEC/09/UCPSc//KU/2019), Kakatiya University, Warangal

4.1.1. Induction of Diabetes in rats:

Wistar rats fasted overnight (180-250gms) and diabetes was induced by administration of Streptozotocin 45mg/kg (i.p) in 0.1M sodium citrate buffer. Rats were immediately administered with 5% dextrose to *antagonise* the rapid hypoglycemia effects. Rats were checked for the blood glucose level after 3 days and rats which had blood glucose level >200mg/dl are included in the study (Akbarzadeh *et al*., 2007).

4.1.2. Pharmacodynamic and Pharmacokinetic interaction study in diabetic rats:

Group 1: Normal control

Group 2: Diabetic control – 45mg/kg STZ given in sodium citrate buffer in (i.p) route.

Group 3: Repaglinide (1mg/kg, p.o) after induction of diabetes.

Group 4: SDT studies-Andrographolide (4.5mg/kg, p.o) followed by Repaglinide (1mg/kg) in diabetes induced rats.

Group 5: MDT Studies- Andrographolide given for 7 days (4.5mg/kg, p.o) and on 8th day Andrographolide (4.5mg/kg, p.o) followed by repaglinide (1mg/kg p.o) in diabetes induced rats.

SDT – Single dose treatment MDT – Multi dose treatment

In pharmacokinetic study, blood samples were collected from retro orbital puncture at time intervals between 0, 0.5,1, 2, 4, 6, 8 and 12 hrs using heparinized capillaries and Serum was separated by centrifugation at 8000 rpm for 15 min and the samples were stored in vials at – 20 °C until further analysis. In pharmacodynamic study, Blood glucose levels were determined using Glucometer method.

4.1.3. Estimation of blood glucose:

There are several methods for the estimation of blood glucose. In the present study we have used electrochemical sensoring using a blood glucose monitoring Glucometer.

Specimen:

The Blood Glucose levels were measured through the blood collected by the retroorbital puncturing and taking a single drop of blood on the test strip using a capillary.

4.2. Pharmacokinetic evaluation:

4.2.1. HPLC description:

A Shimadzu class VP series HPLC system consisted of binary LC-20AD pumps with a micro gradient mixer. RP C18 column, 250 mm×4.6 mm, particle size 5 µm (Phenomenex Luna) was used at 35±2.0 °C. All the operations and analysis of data obtained were controlled by lab solutions software.

4.2.2. Preparation of Standard solutions:

Primary stock solutions of Repaglinide and Gliclazide (Internal standard) were prepared in methanol at a concentration of 1 mg/ml and stored at -200C [Pritosh *et al*., 2013].

4.2.3. Preparation of Mobile phase:

Methanol and water are used in 80:20 ratio.

Standard Graph procedure:

1. Primary stock solution of Repaglinide and Gliclazide (Internal standard) were diluted with methanol:distilled water [mobile phase (80:20)] to obtain the working solutions of 100 µg/ml concentration.

2. From stock solution of Repaglinide, series of dilutions are made to get the concentrations of 0.1, 0.5, 1, 5, 10, 50 µg/mL.

3. To 100 µL of blank serum samples, 100 µL of internal standard from 100 µg/ml of working solution and different concentrations of repaglinide were added to obtain 20 µg/ml final concentration.

4. The resultant solution was mixed for 1 minute on cyclomixer at room temperature and centrifuged at 4000 rpm for 15 min and the supernatant was separated.
5. The supernatant was filtered through 0.2 µm syringe filter, 20 µL of the solution was spiked for the HPLC analysis.
6. Retention time of Repaglinide and gliclazide are 6.2 and 4.18 minutes respectively.
7. The flow rate was 1.0 ml/min and effluent was monitored at 240nm.
8. The peak area of the drug and internal standard was determined, and the peak area ratio was calculated using the formula.

Peak Area Ratio = Peak Area of Drug / Peak Area of Internal Standard

9. Graph was plotted by taking concentration on X-axis and peak area ratio on Y-axis.
10. The standard graph was considered to be significant when the r2 is ≥0.99.

4.2.4. Sample extraction procedure:

To 100 µL of serum samples, 20 µL of internal standard from 100 µg/ml of working solution was added and 100 µL of methanol was added, the resultant solution was mixed for 2 minutes on cyclomixer at room temperature and centrifuged at 4000 rpm for 10 min and the Supernatant was separated and the supernatant was filtered through 0.2 µm syringe filter, 20 µL of the solution was spiked for the HPLC analysis.

4.3.0. Pharmacokinetic analysis

The pharmacokinetic parameters, Peak serum concentrations [C_{max}] and time to reach peak concentration [t_{max}] were directly obtained from concentration-time data. In the present study AUC0-t refers to AUC from 0 to 7 hours, which was determined by linear trapezoidal rule, and AUC0-∞ refers to AUC from 0 to infinity. The AUC0-∞ was calculated using the formula AUC0-t + [Clast/ KCl] where Clast is the concentration in mg/ml at the last time point and KCl is the elimination rate constant.

Various pharmacokinetic parameters like Area under the curve [AUC], Elimination half-life [t1/2], Volume of distribution [Vd], Total clearance [CL/f], and Mean residence time for each sample using a non-compartmental pharmacokinetic program KINETICA based on following equations.

a) Peak serum concentration [C_{max}]

The point of maximum concentration of drug in serum is called as the peak and the concentration of drug at peak is known as peak serum concentration.

b) Time of Peak serum concentration [t_{max}]

The time for the drug to reach peak concentration in the serum is called as time of peak concentration. The above two parameters are obtained from the observed concentration versus time data.

c) Elimination rate constant:

It is the sum of individual rate constants associated with the loss of parent drug from the body. It is a quantitative index of the persistence of drug in the body. This is calculated from the slope of the terminal elimination phase of the logarithmic plot of concentration of the drug in the biological fluid versus time, after subjecting the terminal phase to linear regression analysis.

Slope = n [Σnt=1T1 (log Ci)]-[Σ T1log C1] / n [Σ T12]-[Σ t1]2

Where n= No of points in the terminal phase.

d) Half- life:

Half-life of the drug is defined as the time required to reduce the concentration of drug in the body by 50%. It is determined via the elimination rate constant, assuming to be a first order process.

t1/2= 0.693/K

Where K is the elimination rate constant.

e) Area under the curve [AUC]:

The area under the concentration time curve extended to infinite time represents the bioavailability of a drug. It is determined via the linear trapezoidal rule from 0 to the last sampling time t. It is the area under zero moment curve.

AUC0-t = ∫0t C.dt= Σni=1[(ti+1- ti)/2. (Ci + Ci+1)] conc./ml/h

And for the remaining area (Wagner's approximation)

The total AUC0-∞ = AUC0-t + AUCt-∞ =AUC0-t + Ct / K

Where Ct is the concentration at the last time point.

f) Apparent volume of distribution (Vd):

Once a drug attains distribution equilibrium, and then it exhibits a relation between the concentration of the drug in plasma and the total amount of drug in the body. The proportionality constant relating these two quantities is called the apparent volume of distribution. This is a conventional parameter, which gives an understanding of drug reaching tissue level. However, the volume parameters obtained for any drug administered through any route other than intravenous will never represent the real volume of distribution.

In the present study, the apparent volume of distribution for fraction of drug is calculated by following equation.

V/f = Dose / AUC0-∞ K ml/kg

V = f Dose / AUC0-∞ K ml/kg

Where f is the fraction of the dose absorbed which can be taken as 1.0 for diclofenac based on the earlier reports.

K = Elimination rate constant.

AUC0-∞ = Area under the zero-moment curve.

g) Clearance (Cl):

Systemic clearance or total clearance represents the sum of individual clearances like renal clearance, hepatic clearance and biliary clearance, salivary clearance, and respiratory clearance etc., involved in the elimination of drug in the body. This can be calculated from the expression.

CL/f = Dose / AUC0-∞ ml/h.

Calculations of Pharmacokinetic parameters:

Pharmacokinetic parameters were calculated using "KINETICA" software. All the data were expressed as Mean ± Standard deviation.

5.0. RESULTS AND DISCUSSIONS:

5.1. Results:

5.1.1. Pharmacokinetic study:

Standard graph of Repaglinide in rat serum: The equation of the calibration curve obtained was y=mx+c and its calibration curve were shown below.

Repaglinide Conc.(µg/ml)	IS conc. (µg/ml)	Repaglinide Peak area	IS peak area	Peak Area Ratio (PAR)
0.1	20	3625.1	139426.9	0.026
0.5	20	10784.8	151898.5	0.071
1	20	21125.9	116717.6	0.181
5	20	63591.1	96350.1	0.66
10	20	190765.2	127176.8	1.5
50	20	1018012.0	114512.0	8.89

Table -1: Repaglinide concentrations and PAR in rat serum

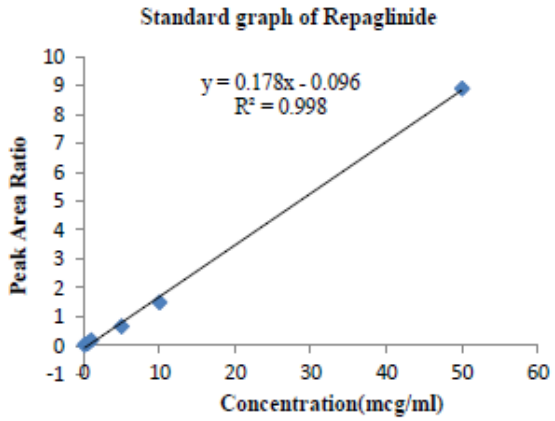

Fig. 2 Calibration curve of Repaglinide in rat serum.

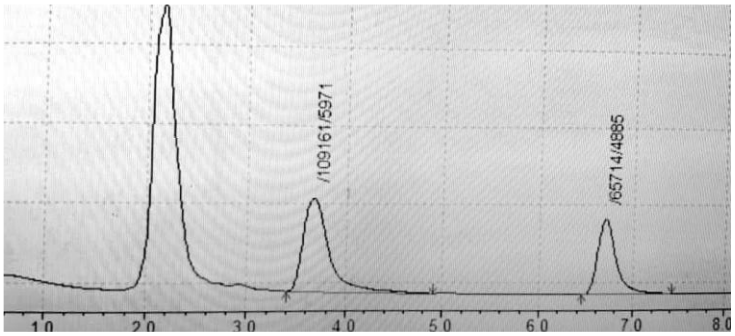

Fig: 3: HPLC chromatogram of Repaglinide and IS

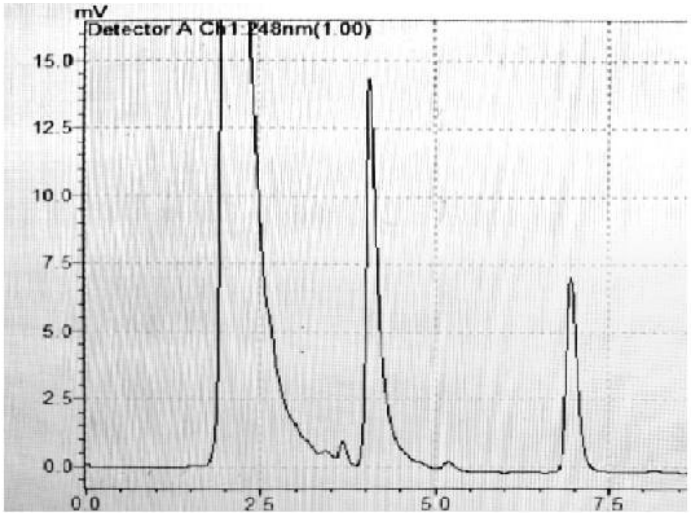

Fig: 4: HPLC chromatogram of Repaglinide and Andrographolide in rat serum

Flow rate: 1.0 ml/min
λ max: 248 nm
Retention time for Repaglinide: 6.2 min
Retention time for IS(Gliclazide): 4.18 min.

Table: 2 Mean serum concentration (µg/ml) of Repaglinide and Repaglinide in presence of Andrographolide (SDT & MDT) in diabetic rats.

Time(hr)	Repaglinide (µg/ml)	Repaglinide+ Andrographolide (SDT)(µg/ml)	Repaglinide+ Andrographolide (MDT)(µg/ml)
0.5	0.459±0.0894	0.733±0.0384**	1.194±0.0304**
1	1.521±0.0706	1.670±0.0447	2.459±0.2317**
2	0.946±0.0393	1.252±0.0429**	2.082±0.1628**
4	0.756±0.0294	0.921±0.1216	1.726±0.1932**
6	0.614±0.0277	0.732±0.0375*	1.133±0.1279**
8	0.428±0.3188	0.676±0.0304**	0.912±0.1896**
10	0.342±0.0196	0.498±0.3757**	0.573±0.0742**
12	0.181±0.0277	0.314±0.0214**	0.479±0.0706**

Mean ± SD: All values are expressed as mean±SD(n=6).** significant at p<0.01; *significant at p<0.05; ns at p>0.05 compared to Repaglinide control; SDT (single dose treatment); MDT (multiple dose treatment). Statistical analysis was performed using one way ANOVA (Dunnet multiple comparision test).

Mean serum concentration (µg/ml) of Repaglinide and Repaglinide in presence of Andrographolide (SDT & MDT) in diabetic rats.

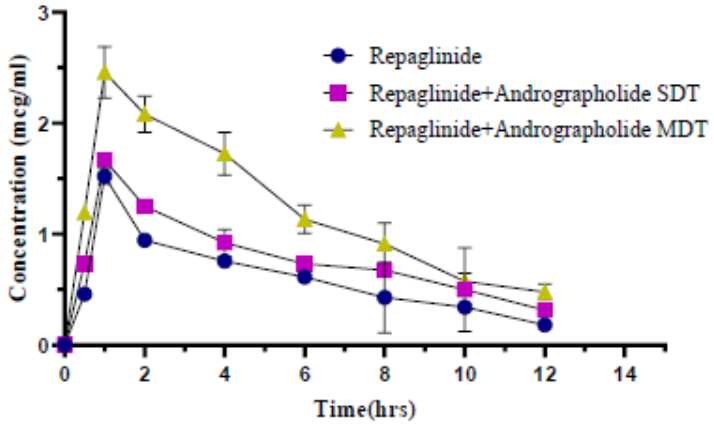

Table: 3: Mean serum concentration (µg/ml) of Repaglinide and Repaglinide in presence of Andrographolide (SDT & MDT) in diabetic rats

PK parameter	Repaglinide	Repaglinide+ Andrographolide (SDT)	Repaglinide+ Andrographolide (MDT)
Cmax (µg/ml)	1.512±0.530	1.690±0.191*	2.681±0.247***
tmax(hrs)	1.00 ±0.231	1.00±0.408	1.00±0.516
AUC0-t (µg/hr ml)	58.12±1.27	77.61±3.69*	102.05±7.01**
AUMC0-t(µg/h*h ml)	127.40±1.55	135.72±6.18**	152.89±2.31***
t1/2(hrs)	1.516±0.362	2.055±1.438	3.261±1.050**
MRT (hrs)	3.572±0.24	4.89±2.74*	6.31±0.812**
Cl(ml/hr)	309.53±1.45	267.0±1.58*	109.03±0.37***
Vd(ml)	978.15±6.86	835.96±7.23**	764.11±5.31***
Vdss(ml)	1407.03±7.45	1068.42±9.63**	858.74±6.82***

Mean ± SD: ***significant at p<0.001; ** significant at p<0.01; *significant at p<0.05 compared to Repaglinide control; SDT (Single dose treatment); MDT (Multiple dose treatment). Statistical analysis was performed using Two-way ANOVA (Tukey's multiple comparison test).

Pharmacodynamic data:

Table:4: Mean blood glucose levels (mg/dl) in diabetic rats after oral administration of Repaglinide and Repaglinide in presence of Andrographolide (SDT & MDT).

Time(hr)	Repaglinide	Repaglinide+ Andrographolide (SDT)	Repaglinide+ Andrographolide (MDT)
0	253.67±4.061	235.51±4.615**	228.23±2.057**
0.5	223.16±3.515	221.62±2.298	205.08±6.762
1	190.01±1.610	169.3±5.747*	139.30± 5.443**
2	197.83±5.170	180.06±2.886	149.82±0.733**
4	210.27±6.924	183.59±1.004**	157.41±1.986**
6	215.63±2.084	189.72±4.197**	172.84±1.029**
8	223.21±2.694	195.00± 5.894**	189.12±4.275**
10	227.40±5.905	199.29±2.916*	189.00±1.489**
12	231.91±1.164	207.53±3.111**	198.67±3.683**

Mean ± SD: ** significant at p<0.01; *significant at p<0.05; ns at p>0.05 compared to Repaglinide control; SDT (single dose treatment); MDT (multiple dose treatment). Statistical analysis was performed using one way ANOVA (Dunnet test).

5.2. DISCUSSIONS:

Diabetes is a chronic metabolic disorder and needs prolonged treatment for maintenance of normal blood glucose levels. Diabetes may precipitate cardiovascular, renal, neurological disorders. Herbal drugs which alter hepatic and intestinal CYPs can modify the bioavailability and clearance of administered drugs. Repaglinide is metabolized by CYP3A4 and CYP2C8, CYP2C9 enzymes (Nielsen *et al*.,2001; Kiran *et al*.,2013). Andrographolide was reported to inhibit CYP enzyme CYP3A4 (Sujatha and Veeresham *et al*., 2016).

The factors which influence the CYP mediated metabolism either directly or indirectly are likely to be prominent for interaction. It may be due to induction or inhibition of CYP enzyme. The inhibition of CYP enzymes can increase the plasma concentration of simultaneously administered drugs and enhance the pharmacological and toxicological effect. Whereas in case of induction of CYP enzymes, can lower the concentration of plasma of simultaneously administered drugs and reduce the therapeutic effect (Jyothi *et al*., 2017).

The influence of Andrographolide on the pharmacokinetics and pharmacodynamics of Repaglinide was studied in streptozotocin induced diabetic rats. In pharmacokinetic interaction study, the pharmacokinetic parameters were changed in single dose treatment groups, but statistically more significant in multiple dose treatment of combination when compared to repaglinide group. In single dose treatment groups (SDT), Cmax ($p<0.05$) of repaglinide and andrographolide was increased when compared to repaglinide group, but it was

significantly increased($p<0.001$) in multi dose treatment group (MDT) of repaglinide and andrographolide when compared to repaglinide group.

There was no change in the tmax of single dose treated and multi dose treated groups of repaglinide and andrographolide when compared to repaglinide group. t1/2 of repaglinide was significantly ($p<0.01$) increased in rats treated with multiple doses of andrographolide and repaglinide (MDT) when compared to repaglinide group, but there was no such significant increase in single dose treatment groups of repaglinide and andrographolide when compared to repaglinide group. In multi dose treatment groups, AUC_{0-t} of repaglinide and andrographolide increased statistically ($p<0.01$) more significant when compared to repaglinide group. In single dose treatment groups, AUC0-t of repaglinide and andrographolide increased statistically($p<0.05$) significant when compared to repaglinide group.

In multi dose treatment groups, AUMC0-t of repaglinide and andrographolide increased statistically ($p<0.001$) more significant when compared to repaglinide group. In single dose treatment groups, AUMC0-t of repaglinide and andrographolide increased statistically($p<0.01$) significant when compared to repaglinide group. Clearance of repaglinide in rats treated with multiple doses of Andrographolide and repaglinide had significantly decreased ($p<0.001$) when compared to repaglinide group. Clearance of repaglinide in rats treated with single dose of Andrographolide and Repaglinide significantly ($p<0.01$) decreased when compared to repaglinide group. Mean residence time of repaglinide in rats treated with multiple doses of Andrographolide and repaglinide decreased significantly ($p<0.01$) when compared to repaglinide group. Mean residence time of repaglinide in rats treated with single dose of Andrographolide and Repaglinide significantly ($p<0.05$) decreased when compared to repaglinide group.

Volume of distribution of repaglinide in rats treated with multiple doses of Andrographolide and repaglinide decreased significantly ($p<0.001$) when compared to repaglinide group. Volume of distribution of repaglinide in rats treated with single dose of Andrographolide and Repaglinide significantly ($p<0.01$) decreased when compared to repaglinide group. Volume of distribution at steady state of repaglinide in rats treated with multiple doses of Andrographolide and repaglinide decreased significantly ($p<0.001$) when compared to repaglinide group. Volume of distribution at steady state of repaglinide in rats treated with single dose of Andrographolide and Repaglinide significantly ($p<0.01$) decreased when compared to repaglinide group. Maximum glucose reduction was observed at 1 hr and glucose reduction was statistically significant in both single dose treatment and multi dose treatment of repaglinide and andrographolide when compared to repaglinide group. The improved pharmacokinetic parameters of Repaglinide was more observed in the multi dose treatment groups of combination, and the improvement of pharmacodynamics was significant in multi dose treatment of combination. This study shows significant influence of Andrographolide when it is used in combination with repaglinide in multi dose studies.

6.0. CONCLUSIONS:

The results showed that repaglinide levels was increased in single dose and multi dose treatment of repaglinide and andrographolide, may be due to metabolic inhibition of CYP3A4 in the presence of Andrographolide. It indicates that occurrence of drug interaction, which may be due to decreased metabolism of repaglinide. The variation is more prominent in multi dose treatment of andrographolide and repaglinide groups. Since, the changes in pharmacokinetics and pharmacodynamics of repaglinide are more pronounced in multi dose treatment groups of combination, it indicates the significance of long-term exposure of Andrographolide in diabetic condition, thus it may apply to diabetic patients under Repaglinide treatment. Hence, the combination has a beneficial effect in diabetic conditions, but special concern has to be observed in diabetic patients in view of the side effects of Repaglinide. Hence the present investigation warrants further studies to find out the relevance of this interaction in human beings and postulates the exact mechanism involved.

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