PHARMACODYNAMIC AND PHARMACOKINETIC INTERACTION OF TERMINALIA PALLIDA WITH GLICLAZIDE IN NORMAL AND DIABETIC RATS

Satyanarayana CH[1]*, Bimalendu Chowdhury[2]

Abstract

The Current study aims to investigate the interaction between traditionally used anti-diabetic agent hydro-alcoholic extract of Terminalia pallida fruits (HATP) and oral hypoglycemic agent gliclazide. Initial dose optimization was performed after administration of 200 mg/kg and 400 mg/kg of extract and determining reduction in serum glucose levels in normal rats. A pharmacokinetic interaction study was performed in both normal and streptozocin induced (55 mg/kg) diabetic rats by administration of gliclazide only or combined with HATP at 400 mg/kg. Single-dose and repeated dose (28 days) pharmacodynamic and pharmacokinetic interaction studies were performed in diabetic rats after co-administration by measuring serum glucose levels and gliclazide levels respectively. Biphasic pharmacokinetic (serum concentration) and pharmacodynamic (reduction in serum glucose levels) profile was demonstrated by gliclazide. HATP demonstrated higher and dose proportionate serum glucose reduction at 400 mg/kg in normal rats. The reduction in serum glucose levels in normal and diabetic rats was significantly higher in the combined group as compared to only gliclazide group. Repeated co-administration showed a higher reduction in serum glucose levels as compared to single time co-administration. On day 28 biochemical parameters are estimated to evaluate effect on oral administration of HATP with gliclazide for 28 days to diabetic animals. The results are shown a significant improvement in dyslipidemia, triglyceride levels and liver functional parameters such as SGOT, SGPT, ALP, and total protein in HATP combination as compared to the vehicle control group. There was a significant variation observed in pharmacokinetic parameters with single dose co-administration in normal, diabetic rats and in repeated dose co-administration in diabetic rats. HATP showed a significant pharmacodynamic and pharmacokinetic interaction with gliclazide, which necessitates caution and dose adjustment in co-administration of gliclazide with HATP to avoid severe hypoglycemia.

Keywords: Diabetes, Terminalia pallida, Drug interaction, Gliclazide, Pharmacokinetics, Pharmacodynamics

[1*,2]Dept. Of pharmacology, Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, India-760010.

***Corresponding Author:** Satyanarayana CH.,
*Research Scholar, Dept. of pharmacology, Roland Institute of Pharmaceutical Sciences, Berhampur-760010 (Odisha), India, Email: satya_pharm@yahoo.com

DOI: 10.48047/ecb/2023.12.si5a.0497

1. INTRODUCTION:

Diabetes mellitus, a prominent non communicable disease arises from an increase in levels of serum glucose either due to damaged pancreatic beta cells leading to reduced secretion of insulin or desensitization of insulin sensitive tissues to insulin. Diabetes and associated complications are a predisposing factors for the development of obesity, metabolic syndrome and cardiovascular disorders. Globally there is an increase in diabetic patients by 3.8 times in the past three decades and the global diabetic population might be 700 million by year 2045 [1]. Diabetes has increased premature mortality by 5% in the past two decades and it is the causative factor for mortality of 1.5 million individuals globally in year 2019 [2]. The current antidiabetic treatment regimen includes insulin, its preparations, oral hypoglycemic agents such as metformin, thiazolidinediones, sulphonylureas, alpha glucosidase inhibitors, dipeptidyl peptidase-4 inhibitors, sodium/glucose co-transporter 2 inhibitors and other injectables like glucagon-like peptide analogs, gastric inhibitory peptide analogs and glucagon like peptide receptor agonists [3]. Although sulfonylureas are associated with adverse effects such as hypoglycemia, weight gain and cardiovascular effects, they are second to metformin in prescription for diabetes [4]. Sulfonylureas bind to the sulfonylurea receptor located on pancreatic beta cell, blocking ATP sensitive potassium channels thus causing the release of insulin.

Herbal preparations are a source of drugs and played a crucial role in the treatment of various disease conditions from ancient times. Even in the current era plants form a major part of alternative and traditional medicine due to their abundant phytomolecules and their multimode pharmacological activities. As per world health organization records there are 21,000 listed medicinal plants, among which 400 are listed for treatment of diabetes [5]. There is an increase in research and prescription interest for plant derived products as nutritional supplements and as drugs especially for treatment of metabolic disorders such as diabetes mellitus [6–8].Concomitant use of herbal preparations and antidiabetic agents may trigger herb-drug interactions (HDI), which can have impact on safety and efficacy of the drug. HDI may arise due to impact of herbs on pharmacokinetics or pharmacodynamics of the drug. Pharmacodynamic interaction may arise due to enhanced secretion of insulin by various phytomolecules and pharmacokinetic interactions may predominantly arise due to impact of herbs on cytochrome P450metabolic machinery [9,10].

The genus of Terminalia herbs around 250 species of the plants, which are highly used used in different traditional systems of medicine. Among these plants T. arjuna,T. chebula and T. pallidaare widely mentioned in Ayurveda, the Indian system of traditional medicine [11]. T. pallida is one among this genus, belongs to Combertaceae family, is abundantly distributed in Tirumal hills of South Eastern ghats[12]. It is known as Hridya in Ayurveda due to its cardiotonic and cardio-protective properties and it is also one of the ingredient in renownened Ayurvedic product Triphala, used for treatment of liver disorders and indigestion [11,13]. Fruits of this plant are traditionally employed for treatment of diabetes mellitus, fruit decoction used to treat diarrhea, peptic ulcers and veneral diseases [11]. The major phytoconstituents of this plant include gallic acid, ellagic acid, gallo-tannic acid, chebulagenic acid, mannitol, etc [13,14]. Pharmacological studies reported antioxidant, antiulcer, cardioprotective, antidiabetic, antiadipogenic, hypolipidemic and hepatoprotective activities in in vitro and in vivo models [11,12,14,15]. As fruits of T. pallida are traditionally used for treatment of diabetes mellitus and due to its cardiotonic properties there might be concomitant administration of it with other antidiabetic agents such as gliclazide. This study aimed to Current study is designed to determine and assess pharmacological interaction between hydroalcoholic fruit extract of T. pallida and gliclazide using rat models.

2. MATERIALS AND METHODS:

2.1. *Drugs and Chemicals:*

All kits used in the study were procured from Coral clinical systems (Goa, India). Gliclazide was obtained as a gift sample from Dr. Reddy's laboratory (Hyderabad, India), Streptozocin was procured from Sisco Research Labs (Mumbai, India), and Terminalia pallida leaf extract was obtained as a gift sample from Laila Impex Pvt Ltd., (Vijayawada, India). All other reagents and chemicals used in this study were of analytical grade and were procured from Merck Millipore (Massachusetts, USA).

2.2. Animals:

Male Wistar rats of 8-10 weeks old (200- 230gm) were procured from Mahaveer enterprises, (Hyderabad, India) and acclimatized for a week. They were maintained under standard laboratory conditions of 22±3°C temperature and 50±15% relative humidity with 12 hours light/12 hours dark cycle. They were provided with a standard pellet

diet (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum.*

2.3. Experimental Design:

2.3.1. Interaction Study in Normal Rats

This study was designed and executed in IV stages. Gliclazide was administered orally at a dose of 2 mg/kg bw after overnight fasting in stage I. Further animals were subjected to blood collection using retro-orbital plexus puncture under mild isoflurane anesthesia at time points- 0.5, 1, 2, 4, 6, 8, 12 and 24h post gliclazide administration. After phase I same animals were allowed to recover for a washout period of one week and used in further stages. In stage II HATP extract was orally administered at a dose of 200 mg/kg bw and blood was withdrawn at the same time points as of stage I. Further animals were administered with HATP extract at dose of 400 mg/kg bw and blood was collected at above time intervals after a wash out period of 7 days in stage III. In stage IV animals were orally administered with HATP extract at 400 mg/kg bw and gliclazide at 2mg/kg bw with 30 minute time gap after a washout period of week and blood samples were collected. Obtained blood samples were subjected to centrifugation at a speed of 5000 rpm for 5 minutes to collect serum. Serum was further utilized for chromatographic analysis and determination of glucose by glucose oxidase (GOD) peroxidase (POD) method.

2.3.2. Interaction Study in Diabetic Rats

Before induction of diabetes mellitus, animals were deprived with feed for 12 h and provided water ad libitum. Streptozotocin was freshly prepared in citrate buffer (pH 4.5) and intraperitoneally injected at a dose of 55 mg/kg bw. To overcome initial phase of hypoglycemia dextrose solution (20%) was intraperitoneally administered 4-6 h of STZ injection. Further animals were provided orally with 50% dextrose solution till 24 h. Diabetes induction was verified by serum glucose estimation of animals after 72 h using GOD-POD method. Animals with 250 mg/dL or higher glucose levels were considered to be diabetic and utilized for further experiments. Diabetic animals were divided in to three groups, group I animals were orally administered with only gliclazide, group II were administered orally with only HATP extract and group III animals were co-administered with HATP extract followed by gliclazide for 28 days. For estimation of gliclazide and glucose levels serum samples were obtained from animals on day 1 and 28 at time points 0.5, 1, 2, 4, 6, 8, 12 and 24 h.

2.4. Chromatography

Gliclazide concentration in serum samples were estimated by high performance liquid chromatograph (Waters, Japan) equipped with variable wavelength programmable UV or photodiode array detector. This reverse phase HPLC system with C8 column (5 µm particle size; 100 mm length x 4.6 mm diameter) was used as stationary phase. Mobile phase was used in this study was 60:40 mixture of phosphate buffer and acetonitrile with isocratic method. Mobile phase flow rate was 1.2 mL/min and effluent was monitored at 229 nm wavelength. Metformin was used as internal standard, gliclazide concentration was determined from ratio of gliclazide peak area and internal standard peak area. Empower software was used for analysis and interpretation of data (16)**.**

2.5. Sample Preparation &Pharmacokinetic Analysis

To 100µL of serum sample (test or standard) 100 µl of internal standard was added and mixed in micro centrifuge tube. To this mixture 200 µl of acetonitrile was added for protein precipitation, resultant mixture was vortexed and centrifuged at 3000 rpm for 5 minutes. Supernatant was collected and filtered through 0.45 µm membrane filter. Resultant filtrate (20 µl) was injected in to HPLC for analysis of gliclazide. Pharmacokinetic analysis was performed by non-compartment analysis using Kinetica 5.0 software.

2.6. Biochemical parameter analysis

After completion of the 28 days study period, all animals were fasted overnight and sacrificed after collection of blood from retro-orbital plexus puncture under mild anesthesia. Serum samples were separated by centrifuging blood samples at 5000 rpm for 5 minutes at 4°C. From these serum samples following biochemical parameters were determined using appropriate kits. The functional parameters are serum HbAlc, Serum triglycerides, total cholesterol, LDL Cholesterol, HDL Cholesterol levels. The liver function parameters like Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP) and Serum Alkaline Phosphatase (ALP) levels

2.7. Statistical Analysis

All data are represented as mean ± SD/SEM, results were analysed by one way or two way analysis of variance (ANOVA) using Graph pad Prism 7.01 software. Results with $p < 0.05$ were considered as statistically significant.

3. RESULTS

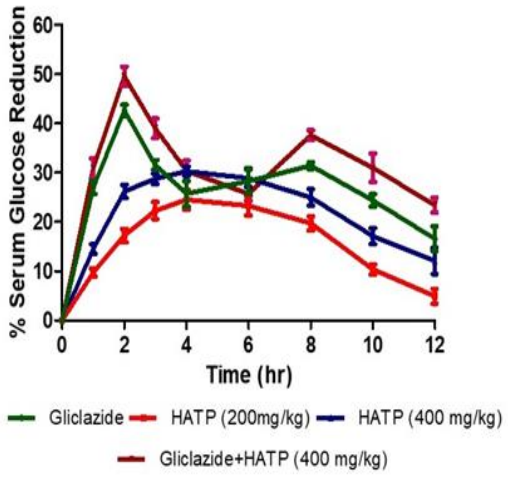
3.1. Pharmacodynamic interaction study in normal rats

Treatment with gliclazide and HATP at 200 & 400 mg/kg bw caused serum glucose level reduction till 12 h (Table 1). Biphasic serum glucose reduction was observed with gliclazide administration. Maximum reduction of 42.62±1.18% and 31.44±0.65% observed at 2h and 8h post administration respectively. Treatment with HATP caused a maximum reduction of 24.54±2.03% and 30.29±0.92% respectively with 200 mg/kg and 400 mg/kg bw at 4h. Co-administration of gliclazide with HATP 400 mg/kg bw caused significantly higher ($p<0.001$) reduction in serum glucose levels as compared to gliclazide only treatment with biphasic reduction of 49.57±1.97% at 2h and 37.620±0.99% at 8h (Figure 1).

Table 1: Serum glucose levels in normal rats treated with gliclazide, *Terminalia pallida* (HATP) 200 and 400 mg/kg and their combination. Data (n=6) was represented as Mean±SEM, analyzed by two way ANOVA and $p < 0.05$ was considered to be significant. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to gliclazide.

Time (h)	Serum Glucose levels (mg/dL)			
	Gliclazide (1mg/kg)	HATP (200mg/kg)	HATP (400mg/kg)	Gliclazide+ HATP (400mg/kg)
0	81.17±1.04	81.50±1.85	84.50±2.51	84.67±2.92 ^{ns}
1	62.33±0.73	77.17±2.12	74.83±2.09	61.17±2.34 ^{ns}
2	**53.90±0.47**	71.50±1.78	67.33±2.11	**48.00±2.67** **
3	61.00±0.94	69.00±2.00	64.33±2.43	57.67±1.76 **
4	64.83±1.28	**64.17±2.05**	**60.17±2.18**	59.83±2.00*
6	62.50±1.35	65.33±2.34	62.00±2.10	64.16±2.11^{ns}
8	**59.67±0.92**	68.67±1.62	67.17±2.15	**55.26±1.37****
10	65.83±0.72	72.67±1.64	72.00±1.85	61.16±1.02**
12	72.33±1.29	76.83±1.40	75.50±1.67	65.50±1.28***

Figure 1: Percent Serum glucose reduction in normal rats treated with gliclazide, *Terminalia pallida fruit* hydroalcoholic extract (HATP) 200 and 400 mg/kg and their combination. Data (n=6) was represented as Mean±SEM.



3.2. Chromatography

The calibration curve for gliclazide in rat serum was linear in concentration range of 0.1 to 100 µg/ml (Figure 2). Lower limit of quantification (LLOQ) for gliclazide was 0.5 µg/ml, chromatogram of gliclazide with internal standard is provided in Figure 3.

Figure 2: Calibration curve for gliclazide in rat serum

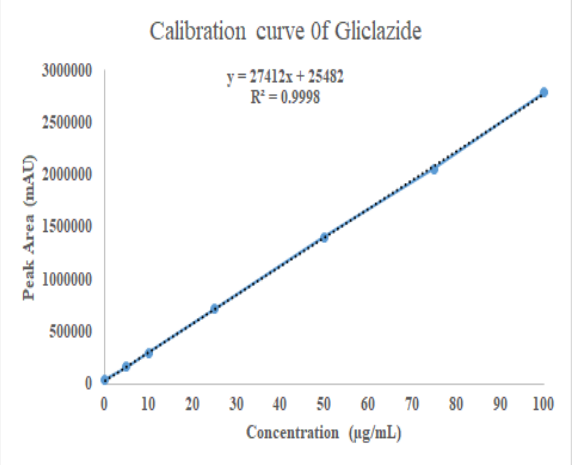
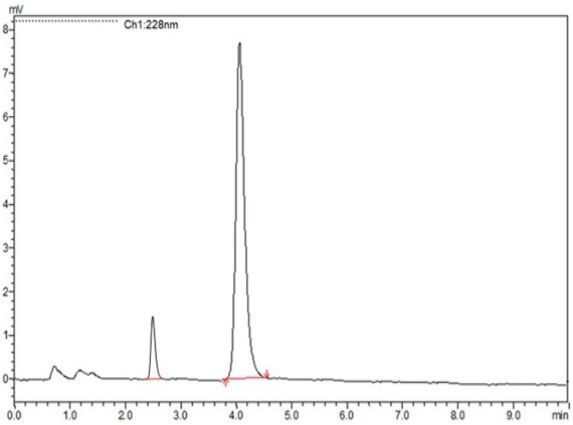


Figure 3: HPLC chromatogram of gliclazide with internal standard in rat serum



3.3. Pharmacokinetic interaction study in normal rats

As pharmacodynamic studies in normal rats demonstrated dose dependent reduction in serum glucose levels, for further studies HATP at 400 mg/kg bw was chosen. Biphasic concentration time data was observed with gliclazide and Cmax was 11.04±0.31 µg/ml at 2h and second spike of 9.05±0.39 µg/ml observed at 8h. Co-administration of gliclazide with HATP caused significant increase ($p<0.05$) in gliclazide concentration through all time intervals as compared with only gliclazide treated group and Cmax was 14.19±0.61 µg/ml. Pharmacokinetic parameters were significantly ($p<0.001$) increased; Area under curve (AUC0-inf) increased by 1.17 times, Mean residence time (MRT) by 1.18 times, elimination half-life (T1/2) by 1.22 times whereas volume of distribution (Vd) increased non significantly by 1.07 times in combined treatment as compared to gliclazide only treatment. Clearance decreased ($p<0.001$) significantly by 1.30 times in combined group as compared to gliclazide only group. Serum gliclazide concentration time profiles of all groups are depicted in Figure 4 and determined pharmacokinetic parameters are provided in Table 2.

Figure 4: Effect of HATP (400 mg/kg) co-administration on serum gliclazide concentration in normal rats. Data (n=6 was represented as Mean±SD**.**

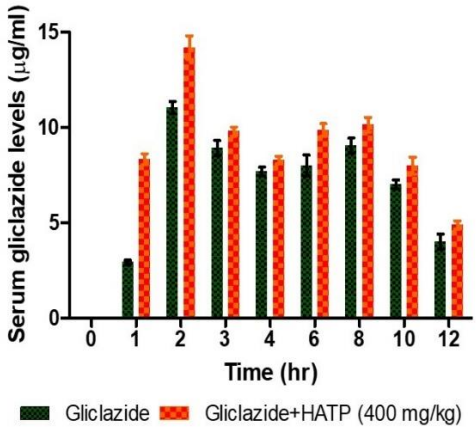


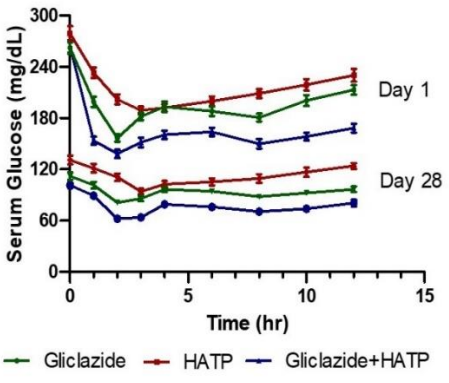
Table 2: Effect of HATP (400 mg/kg) co-administration on pharmacokinetic parameters of gliclazide in normal rats. Data (n=6) was represented as Mean±SD, analyzed by two way ANOVA and $p < 0.05$ was considered to be significant. $^{*}p<0.05$, $^{***}p<0.001$ when compared to gliclazide.

PK Parameter	Gliclazide	Gliclazide + HATP (400mg/kg)
AUC_{0-t}(µg/ml/h)	85.86±1.08	96.99±1.23***
AUC_{total}(µg/ml/h)	101.32±1.92	118.12±2.06***
$T_{1/2}$(h)	2.99±0.14	3.65±0.10
Clearance (L/h/kg)	0.074±0.00	0.057±0.00***
V_d(ml/kg)	0.082±0.00	0.088±0.00
MRT (h)	7.63±0.29	8.97±0.31***
C_{max} (µg/ml)	11.04±0.16	12.10±0.10 **
T_{max}(h)	2.00±0.00	2.00±0.00

3.4. Pharmacodynamic interaction study in diabetic rats

Induction of diabetes mellitus by STZ has caused significant increase in serum glucose levels of the animals. Gliclazide treatment has significantly reduced serum glucose levels as compared to base levels. In diabetic animals also there was biphasic serum glucose reduction with maximum reduction at 2h followed by 8h. Maximum reduction in blood glucose level observed was 1.67 times at 2h. Single dose administration of HATP also caused a reduction in blood glucose levels with maximum reduction of 1.48 times at 3h. Simultaneous administration of HATP and gliclazide has caused significantly higher reduction in blood glucose levels as compared to gliclazide only group with a maximum reduction of 1.91 times at 2h post administration. Repeated administration of HATP for 28 days has caused a significant reduction in the blood glucose levels of animals as compared to day 1. Simultaneous administration of HATP and gliclazide to diabetic animals has caused higher reduction in blood glucose levels as compared to gliclazide only group (Figure 5).

Figure 5: Effect of gliclazide, HATP 400 and their combination on serum glucose levels in diabetic rats on day 1 and day 28



3.5. Pharmacokinetic interaction study in diabetic rats

Similar to normal animal concentration-time data, there was biphasic concentration-time profile observed for gliclazide in diabetic rats. Single dose administration of HATP caused a significant ($p<0.01$) increase of 1.16 times and repeated dose administration of HATP for 28 days caused a significant ($p<0.001$) increase of 1.50 times in Cmax. There was a significant ($p<0.001$) variation observed in all major pharmacokinetic parameters with single and repeated administration of HATP with gliclazide. AUCtotal increased by 1.30 times, T1/2 by 1.50 times, and the Vd by 1.19 times, MRT by 1.19 times and clearance decreased by 1.24 times with single dose administration. Whereas with repeated dose administration AUCtotal increased by 1.67 times, T1/2 by 1.72 times, Vd by 1.43 times, MRT by 1.40 times and clearance decreased by 2.00 times. Serum gliclazide concentration time profiles of all groups are showed in Figure 6 and determined pharmacokinetic parameters are provided in Table 3.

Figure 6: Effect of HATP (400 mg/kg) co-administration on serum gliclazide levels in diabetic rats on day 1 and day 28

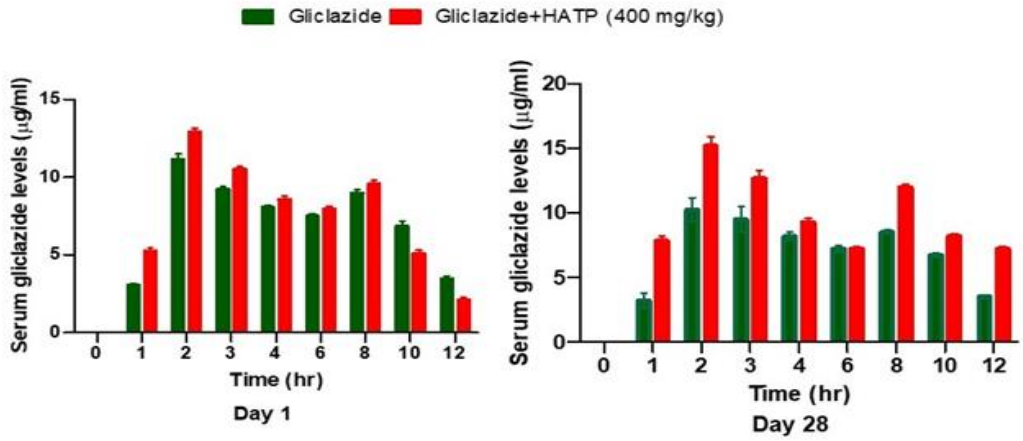


Table 3: Effect of HATP (400 mg/kg) co-administration on pharmacokinetic parameters of gliclazide in diabetic rats on day 1 and day 28. Data (n=6) was represented as Mean±SD, analysed by two way ANOVA and $p < 0.05$ was considered to be significant. $^{*}p<0.05$, $^{***}p<0.001$ when compared to gliclazide.

PK Parameters	Day 1		Day 28	
	Gliclazide	Gliclazide + HATP (400mg/kg)	Gliclazide	Gliclazide +HATP (400mg/kg)
AUC_{0-t}(µg/ml/h)	84.31±1.10	95.68±0.99***	85.19±1.27	111.65±2.08***
AUC_{total}(µg/ml/h)	98.79±1.47	128.20±2.03***	101.27±2.39	169.08±3.86***
$T_{1/2}$(h)	2.94±0.37	4.42±0.23	3.19±0.19	5.50±0.31***
Clearance (L/h/kg)	0.073±0.00	0.059±0.00	0.070±0.00	0.035±0.00***
V_d(ml/kg)	0.072±0.00	0.086±0.00	0.076±0.00	0.109±0.00***
MRT (h)	7.62±0.23	9.06±0.49*	7.60±0.09	10.68±0.07***
C_{max} (µg/ml)	11.28±0.48	12.93±0.61	10.75±0.1	15.72±0.15***
T_{max}(h)	$2±0^{ns}$	$2±0^{ns}$	$2±0^{ns}$	$2±0^{ns}$

3.6. Biochemical parameters estimation in diabetic rats post 28 days

After completion of the 28 days study period, all animals were fasted overnight and sacrificed after collection of blood from retro-orbital plexus puncture under mild anesthesia. Serum samples were separated by centrifuging blood samples at 5000 rpm for 5 minutes at 4°C. From these serum samples following parameters were determined. Data (n=6) was represented as Mean ± SEM.

a. Effect of HATP on Glucose levels and Glycated hemoglobin

Glycated haemoglobin concentration and fasting serum glucose levels were increased with vehicle treatment for 28 days after STZ administration. There was a significant glucose reduction with ***$p<0.001$ observed after treatment with gliclazide, HATP and combination with respect to vehicle treatment.

Figure 7: Effect of Gliclazide, HATP and combination treatments on serum glucose levels and glycated haemoglobin (HbA1c).

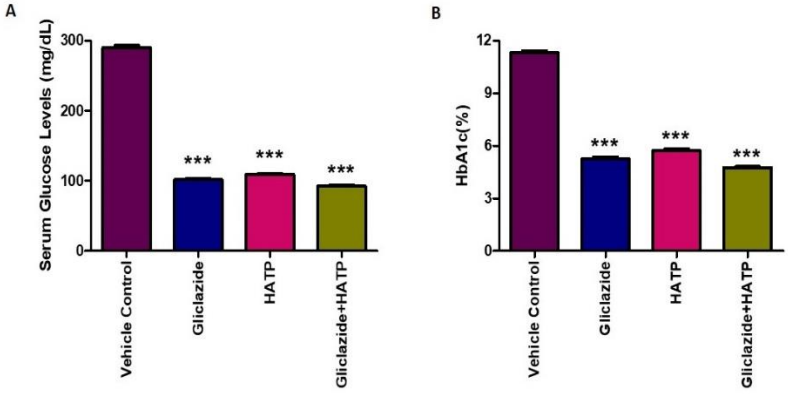


Table 4: Effect of Gliclazide, HATP and combination treatments on serum glucose levels and glycated haemoglobin (HbA1c). Data (n=6) was represented as Mean ± SEM.

S. No	Group	Serum Glucose levels (mg/dL)	Glycated hemoglobin (%)
1	Vehicle control	290.06 ± 8.15	11.31 ± 0.28
2	Gliclazide	101.65 ± 4.19	5.26 ± 0.25
3	HATP	109.26 ± 3.60	5.71 ± 0.29
4	HATP + Gliclazide	92.52 ± 3.53	4.77 ± 0.11

b. Effect of HATP on Triglycerides and Cholesterol levels

Administration of STZ and treatment of animals with vehicle for 28 days caused increase in lipid parameters: total, LDL cholesterol, triglyceride levels and decline in HDL cholesterol levels. Treatment with gliclazide, HATP and combination to diabetic animals led to amelioration of total, HDL, LDL cholesterol and triglyceride levels as compared to vehicle control group with ***$p<0.001$ significance.

Figure 8: Effect of Gliclazide, HATP and combination treatments on serum triglycerides and cholesterol Mean ± SEM.

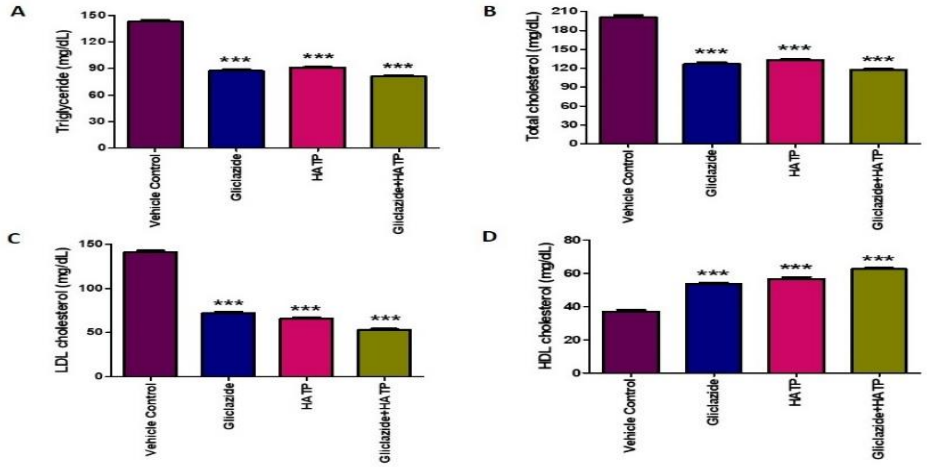


Table 5: Effect of Gliclazide, HATP and combination treatments on serum triglycerides and cholesterol. Data (n=6) was represented as Mean ± SEM.

Group	Vehicle control	Gliclazide	HATP	Gliclazide + HATP
Triglycerides	143.30 ± 4.17	87.29 ± 4.18	91.25 ± 2.07	81.21 ± 2.56
Total Cholesterol	201.16 ± 7.32	126.85 ± 6.97	133.00 ± 4.70	117.38 ± 5.11
LDL- Cholesterol	141.18 ± 5.31	72.04 ± 4.58	65.87 ± 4.09	53.25 ± 2.65
HDL- Cholesterol	36.94 ± 2.63	53.79 ± 1.84	56.57 ± 3.30	62.61 ± 2.24

c. Effect of HATP on Liver Functional Parameters

There was a significant augmentation observed in liver functional parameters serum ALP, SGPT, SGOT, and total protein levels after STZ administration and treatment with vehicle for 28 days indicating damage to liver. Treatment with HATP, gliclazide and combination of these two treatments for 28 days caused a significant (***p<0.001) improvement in these parameters indicating their hepatoprotective nature.

Figure 9: Effect of Gliclazide, HATP and combination treatments on serum liver markers Mean ± SEM**.**

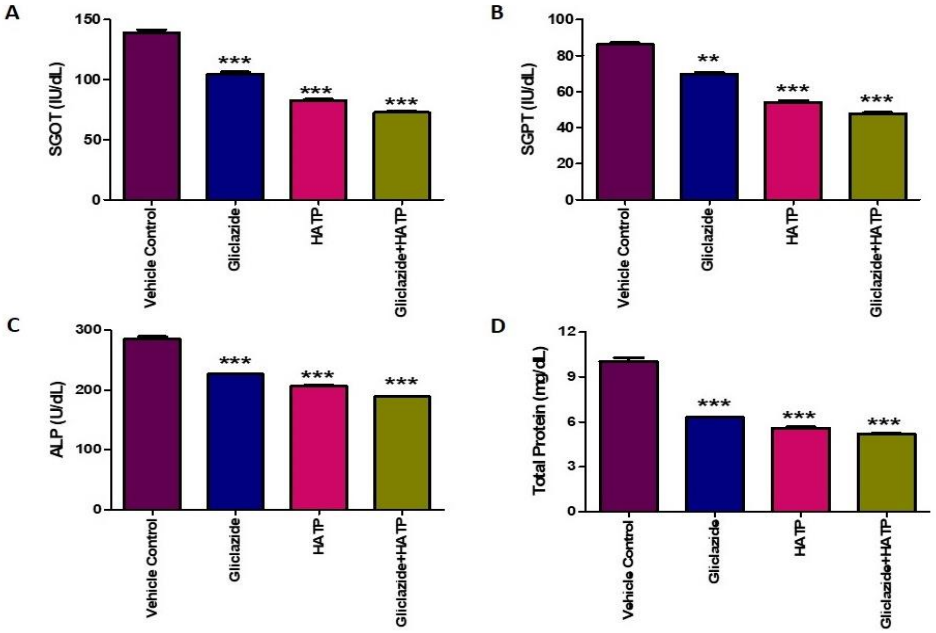


Table 6: Effect of Gliclazide, HATP and combination treatments on serum liver markers. Data (n=6) was represented as Mean ± SEM.

Group	Vehicle control	Gliclazide	HATP	Gliclazide + HATP
SGOT	139.16 ± 5.57	104.49 ± 4.66	82.66 ± 2.59	72.69 ± 3.04
SGPT	86.17 ± 2.51	69.85 ± 2.43	54.14 ± 2.37	47.89 ± 1.39
ALP	285.33 ± 9.93	226.61 ± 2.49	206.96 ± 4.03	188.84 ± 2.94
Total Protein	10.04 ± 0.63	06.31 ± 0.12	05.59 ± 0.23	05.19 ± 0.15

4. DISCUSSION:

Concomitant usage of medicines from traditional systems of medicine is predominant factor for drug interactions as they will be used without consulting with physicians [17]. As drugs from traditional medicine are considered safe they will be used without prescription and especially in case of chronic metabolic disorders there is more possibility of co-administration of medicines from other systems along with antidiabetic drugs. In this study we evaluated herb drug interaction of gliclazide and fruits of Terminalia pallida, which is traditionally used for treatment of diabetes. Study results depicted biphasic pharmacokinetic and pharmacodynamic profile of gliclazide in both diabetic and normal animals due to enterohepatic recirculation and biliary excretion as observed in literature [18]. Initially HATP was administered at 200 and 400 mg/kg bw doses for dose optimization and test efficacy in normal rats. Results of the demonstrated dose dependent hypoglycemic potential of HATP and from this data 400 mg/kg dose was used for further experiments. There was a significant increase in blood glucose reduction effect of gliclazide observed in single and repeated dose co-administration of HATP in normal and diabetic rats, which might be due to pharmacodynamics and or pharmacokinetic interaction. HATP only administration showed reduction in blood glucose levels indicating pharmacodynamic drug-herb interaction between gliclazide and HATP. Role of pharmacokinetic interaction was further studied by determining pharmacokinetic parameters in normal and diabetic animals after co-administration. There was significant increase in serum concentrations of gliclazide at all the time points and significant variation in major pharmacokinetic parameters

such as area under curve, half-life, clearance and volume of distribution in single dose co-administered group as compared to gliclazide group. Similar results were observed even in diabetic animals with single dose of HATP co-administration. Repeated dose administration of HATP depicted higher variation in the serum gliclazide levels and pharmacokinetic parameters as compared to single dose administration. These results suggest involvement of both pharmacokinetic and pharmacodynamic herb-drug interaction between HATP and gliclazide. Pharmacokinetic interaction may not involve absorption phase for gliclazide as it has rapid oral absorption [19]. Gliclazide is extensively metabolized in to inactive metabolites by CYP2C9 and 2C19, induction or inhibition of these enzymes will have significant impact on its serum levels and pharmacokinetics [20]. Plant based treatments might have possible effect on CYP450 metabolic machinery due to their ubiquitous components, which might cause pharmacokinetic interactions and drug herb interactions [17]. Gallic acid one of the major phytoconstituents has inhibitory effect on CYP3A4 metabolic machinery and several studies reported metabolic inhibitory potential of Terminalia arjuna, another plant from same genus [21,22]. These data suggest a possible metabolic machinery inhibition by HATP, which might be causing pharmacokinetic herb-drug interaction.

Serum biochemical parameters were studied to determine liver function, hyperlipidemia and to evaluate the possible effect of the treatments. The hyperglycemia has caused a substantial upsurge in serum triglyceride, cholesterol (Total, LDL) and a significant decrease in HDL cholesterol as represented in vehicle control group animals. Treatment with gliclazide, HATP, and combination treatments have caused improvement of dyslipidemia caused by hyperglycemia. Similarly treatments have caused a significant reduction in the serum SGOT, SGPT, ALP, and total protein levels indicating the possible hepato-protective effect with HATP and gliclazide treatments.

5. CONCLUSION:

Current study results demonstrate hypoglycemic potential of HATP and enhanced hypoglycemic effect of gliclazide when co-administered once or repeatedly with HATP in normal and diabetic rats. Our Study also demonstrated significant rise in gliclazide serum levels in normal and diabetic rats after HATP co-administration in single or multiple doses. Inhibition of CYP based metabolic machinery by gallic acid and other components of HATP might be responsible for increase in serum gliclazide levels, thus causing pharmacokinetic interaction. Study results conclude that HATP has pharmacokinetic and pharmacodynamics interaction with gliclazide thus causing hypoglycemia with co-administration. Oral co-administration of HATP along with gliclazide for 28 consecutive days to diabetic animals caused a significant improvement in dyslipidemia, triglyceride levels and liver functional parameters such as SGOT, SGPT, ALP, and total protein as compared to vehicle treatment. So, precautions has to be taken and dose adjustments has to be performed by patients when HATP is used in alternative systems of medicine in diabetic patient undergoing treatment with gliclazide

6. ETHICAL APPROVAL

The experiments were approved by Institutional Animal Ethical Committee, Roland Institute of Pharmaceutical Sciences, Berhampur (926/PO/Re/S/06/CPCSEA) and conducted as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

7. CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES:

1. Thikekar AK, Thomas AB, Chitlange SS. Herb-drug interactions in diabetes mellitus: A review based on pre-clinical and clinical data. Phytother Res. 2021 Sep;35(9):4763–81.
2. World Health Organization. Diabetes: https://www.who.int/news-room/fact-sheets/detail/diabetes
3. Artasensi A, Pedretti A, Vistoli G, Fumagalli L. Type 2 Diabetes Mellitus: A Review of Multi-Target Drugs. Molecules. 2020 Apr 23;25(8):1987.
4. Andr J Scheen. Sulphonylureas in the management of type 2 diabetes: To be or not to be?. Diabetes Epidemology and management. 2021 January-March; 1; 1-8.
5. Kumar S, Mittal A, Babu D, Mittal A. Herbal Medicines for Diabetes Management and its Secondary Complications. Curr Diabetes Rev. 2021;17(4):437–56.
6. Gupta RC, Chang D, Nammi S, Bensoussan A, Bilinski K, Roufogalis BD. Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and clinical implications. DiabetolMetabSyndr. 2017 Jul 26;9:59.

7. A. Lingesh, D. Paul, V. Naidu, N. Satheeshkumar. AMPK activating and anti adipogenic potential of Hibiscus rosasinensis flower in 3T3-L1 cells. J Ethnopharmacol. 2019 April; 233:123-130
8. Zafar A, Alruwaili NK, Panda DS, Imam SS, Alharbi KS, Afzal M, et al. Potential of Natural Bioactive Compounds in Management of Diabetes: Review of Preclinical and Clinical Evidence. CurrPharmacol Rep. 2021 Jun 1;7(3):107–22.
9. Wanwimolruk S, Prachayasittikul V. Cytochrome P450 enzyme mediated herbal drug interactions (Part 1). EXCLI J. 2014 Apr 2;13:347–91
10. Kandukoori NR, Uppu P, Yellu NR. Study of alterations in pharmacokinetics and pharmacodynamics of Saxagliptin in the presence of Rutin: An interaction study in rats. Journal of Applied Pharmaceutical Science. 2020; 10 (11) :81-86.
11. A.H. Shaik, S.N. Rasool, A. Vikram Kumar Reddy, M. Abdul Kareem, G. Saayi Krushna, K. Lakshmi Devi, Cardioprotective effect of HPLC standardized ethanolic extract of Terminalia pallida fruits against isoproterenol-induced myocardial infarction in albino rats, J. Ethnopharmacol. 2012; 141:33–40.
12. B. Kameswara Rao, P. Renuka Sudarshan, M.D. Rajasekhar, N. Nagaraju, Ch. Appa Rao, Antidiabetic activity of Terminalia pallida fruit in alloxan induced diabetic rats, J. Ethnopharmacol. 2003; 85:169–172.
13. Nagarani K., Swathi P., Eswar Kumar Kilari. Comparative antioxidant activity of selected flower extracts: An *in vitro* study. Journal of Natural Remedies. 2020; 21: 150-161
14. S. Althaf Hussain, M.A. Kareem, S.N. Rasool, S.Y. Al Omar, A. Saleh, M.A. Al-Fwuaires, J.R. Daddam, K.L. Devi, Trace Element Determination and Cardioprotection of Terminalia pallida Fruit Ethanolic Extract in Isoproterenol Induced Myocardial Infarcted Rats by ICP-MS, Biol. Trace Elem. Res. 2018; 181:112–121.
15. M. Gupta, U.K. Mazumder, L. Manikandan, S. Bhattacharya, G.P. Senthilkumar, R. Suresh, Anti-ulcer activity of ethanol extract of Terminalia pallida Brandis. in Swiss albino rats, J. Ethnopharmacol. 2005; 97:405–408.
16. Vatsavai LK, Kilari EK. Interaction of p-synephrine on the pharmacodynamics and pharmacokinetics of gliclazide in animal models. Journal of Ayurveda and Integrative Medicine. 2018 Jul 1;9(3):183–9
17. Asher GN, Corbett AH, Hawke RL. Common Herbal Dietary Supplement—Drug Interactions. AFP. 2017 Jul 15;96(2):101–7.
18. S.K. Mastan, K. Eswar Kumar. Influence of atazanavir on the pharmacodynamics and pharmacokinetics of gliclazide in animal models. International journal of diabetes melitus. 2010; 2(1): 56-60
19. Sarkar A, Tiwari A, Bhasin PS, Mitra M. Pharmacological and Pharmaceutical Profile of Gliclazide: A Review. Journal of Applied Pharmaceutical Science.2011; 01(09):11-19.
20. Shao H, Ren XM, Liu NF, Chen GM, Li WL, Zhai ZH, et al. Influence of CYP2C9 and CYP2C19 genetic polymorphisms on pharmacokinetics and pharmacodynamics of gliclazide in healthy Chinese Han volunteers. J Clin Pharm Ther. 2010 Jun;35(3):351–60.
21. Pu, Qiang-Hong, Shi, Liang, Yu, Chao. Time-dependent inhibition of CYP3A4 by gallic acid in human liver microsomes and recombinant systems, Xenobiotica Fate Foreign Compd. Biol. Syst. 2015: 45: 213–217.
22. A. Varghese, J. Savai, N. Pandita, R. Gaud, In vitro modulatory effects of Terminalia arjuna, arjunic acid, arjunetin and arjungenin on CYP3A4, CYP2D6 and CYP2C9 enzyme activity in human liver microsomes. Toxicol. Rep. 2015:806–816.