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Original Research Article

Effect of herbal formulation on glimepiride pharmacokinetics and pharmacodynamics in nicotinamide-streptozotocin-induced diabetic rats

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ABSTRACT

Background: Traditional medicinal herbs are widely consumed in developing countries to treat diabetes as they are perceived to be safer, less expensive, and have fewer side effects as compared to the conventional medicines. Diabecon (DB), Himalaya Herbal Healthcare, India is herbal over-the-counter formulation which contains several herbs that are reported in the traditional texts for the treatment of diabetes. The majority of these herbs have been investigated and found to interfere with the cytochrome pathway. The most common oral antihyperglycemic drug used today in clinical practice is Glimepiride (GP).The CYP2C9 enzyme is mainly responsible for the metabolism of GP. Herein we hypothesize that the co-administration of GP with DB may result in possible Herb–Drug Interactions (HDIs) as DB has the potential to significantly inhibit the CYP2C9 enzyme.
Objective: In the current study, the pharmacokinetic and pharmacodynamic interactions of GP (0.82 mg/kg) with DB (110.95 mg/kg) was investigated in diabetes induced (Nicotinamide-STZ) rats by co-administering both drugs orally for 21 days.
Materials and methods: For the study of the HDI, Bioanalytical RP-HPLC/PDA method for quantifying GP in plasma of rats was developed and validated as per US-FDA guidelines. *In vivo* pharmacokinetic and pharmacodynamic parameters were studied on day 1 and day 21 post administration.
Results: The RP-HPLC/PDA method was successfully employed for quantification of GP in the PK studies. The co-administration of GP and DB in diabetic rats resulted in beneficial pharmacodynamic interactions, but there were no notable changes in the pharmacokinetic parameters of GP.
Conclusion: This current investigation in an animal model suggests that co-administration of GP and DB may have significant therapeutic benefits in the treatment of diabetes; however, additional research, randomized clinical trials or case studies in humans, is needed.

1. Introduction

Over the past few years, the diabetes mellitus epidemic has rapidly spread worldwide. By 2030, diabetes mellitus will affect 10.2% (578 million) of the global population, rising to 10.9% (700 million) by 2045. In the last several years; China, India, the United States of America, Pakistan, and Brazil have been the epicenters of diabetes mellitus [1]. Today, around 60% of the world's population consumes medicines derived from traditional medicinal herbs [2]. The World Health Organization (WHO) has hoarded a list of 21,000 medicinal plants used around the world. Of these, there are more than 2000 species found in India, about 10% of them being used commercially [2]. In developing countries, herbal formulations (mixture of traditional herbal medicines) are being employed for the treatment of diabetes due to the widespread perception that its safe, as well as they are easily available in natural habitats and are less expensive than the majority of the drugs used in diabetes care [3]. Herbal formulations are made up of a variety of plant mixtures that contain pharmacologically active phytochemicals like

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alkaloids, steroid etc. This complexity raises the possibility of clinical herb–drug interactions (HDIs) [4]. These traditional medicinal herbs may function as inducers or inhibitors of cytochrome P450, efflux and transport proteins, or both, modifications in gastrointestinal and renal functions resulting in HDIs [5].

The mechanism of action of a combination is unlikely to be as important as that of the individual medications [6,7]. However, the latest studies showed that there exist interactions between some commonly administered traditional medicinal herbs and widely used oral hypoglycemic agents in diabetes mellitus [8–15]. Thereby, efforts should be made to identify the therapeutic potential of herbs, assessment of the toxicity, efficacy and mode of action in HDIs to promote their safe and efficient use. One of the main mechanisms of HDIs is the inhibition or induction of the metabolizing enzymes. HDIs are mainly caused by members of the cytochrome P450 superfamily 1, 2, and 3 [16]. Oral hypoglycemic agents are metabolized by mainly CYP2C9, CYP2C19, CYP2C8, CYP3A4 and CYP2D6 enzymes. In the diabetic patient's inhibition or induction of the metabolizing enzymes by herbals may cause the risk of toxicity or undesirable HDIs [17]. In a recent survey conducted, it was observed that the majority of the patients with diabetes mellitus were consuming prescribed modern medicine GP along with herbal formulation DB without a prescription [18]. Traditional herbs can be used as an adjuvant therapy in diabetes treatment, but the patient may still need to take normal antidiabetic medications to keep their blood sugar under control [19]. The herbal formulation DB, is a patented Ayurvedic medicine and phytomineral preparation in tablet dosage form consisting of a blend of several herbs/extracts and minerals well known for its traditional use as antidiabetic through effective glucose utilization, promotion of insulin production in the pancreas, regeneration of pancreatic β-cells, detoxifier, regulation of lipid and cholesterol synthesis, rejuvenator, hepatoprotective as mentioned in Indian Ayurvedic Pharmacopeia and The Indian Materia Medica as shown in Table 1 [20–27]. The majority of the components have also been studied through suitable *in vitro/in vivo* models for their potential to significantly inhibit the CYP (2C9, 1A2, 3A4, 2D6, 2C19, 2D9, 2B6) [28–36].

GP is primarily metabolized by the CYP2C9 enzyme. Increased plasma GP concentration due to HDI can cause severe hypoglycemia, with seizures, coma, or other neurological problems [17]. Various methodologies for GP bio analysis have been established. In the current investigation, an attempt was made to use established methods to determine GP concentration in rat plasma following a PK based HDI study involving herbal formulation diabecon (DB). However, due to matrix interferences from both plasma and the herbal formulation, the reported methods were unable to accurately estimate GP. It was not possible to expand the existing methods for this particular application. Hence, in the present investigation, a sensitive and simple bioanalytical RP-HPLC/PDA method was developed for the determination of GP in plasma of rat and validated (US-FDA guidelines) for the HDI study of DB and GP.

Hence we postulate that the co-administration of GP with herbal formulation DB may lead to potential HDI. In the current study, the potential HDI was estimated by investigating the effect of DB on the *in vivo* pharmacokinetic and pharmacodynamic behavior of GP by co-administration via oral route in diabetes induced (Nicotinamide-STZ) Wistar rats.

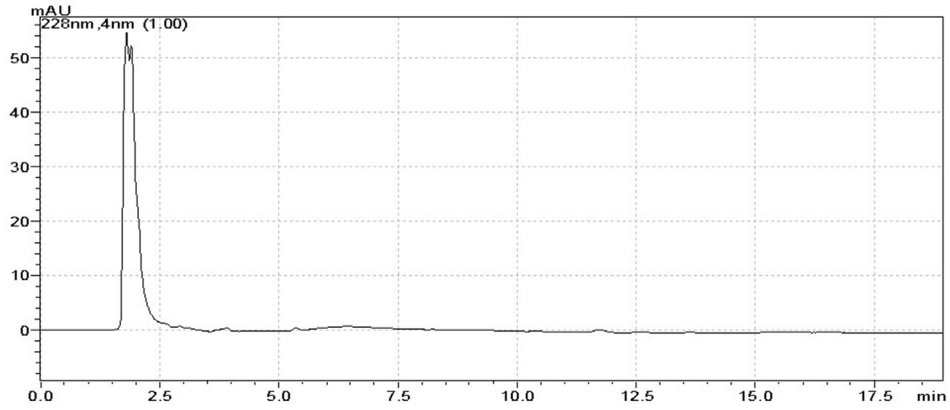
2. Materials and methods

2.1. Drugs and chemicals

Herbal formulation DB (Himalaya Herbal Healthcare) was purchased from the local Ayurvedic store (Table 1). GP (purity ≥99.5%) and Gliclazide (purity ≥99.5%) (Internal standard, IS) was provided as gift samples from IPCA Laboratory Limited, Kandivali West, Mumbai, India. Acetonitrile (HPLC grade) was supplied by Merck Chemicals, Mumbai, India. Millipore water purification system was

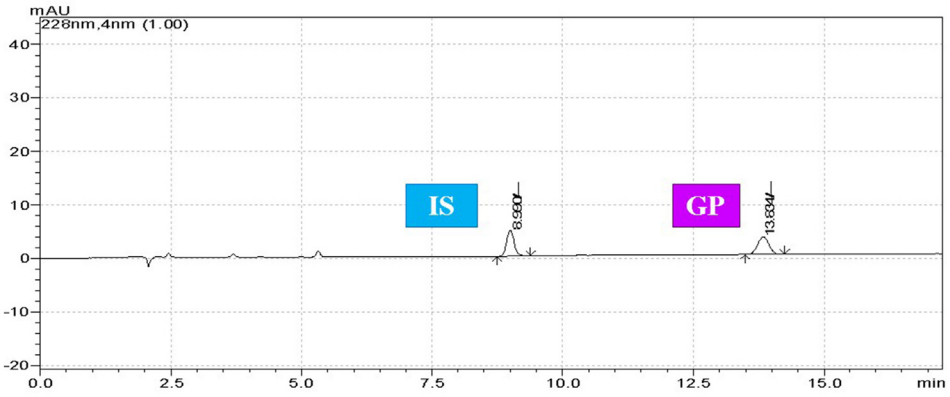
Table 1
Key Ingredients of Diabecon formulation.

Ingredients	Actions	Quantity
Commiphora Wightii (Arn.) Bhand.-Shuddha Guggul-Indian Myrrh [23]	Anti-diabetic, Antioxidant	30 mg
Asphaltum punja-Bianum-Purified Shilajit-Mineral pitch [22]	Rejuvenation, Anti-aging, Detoxification, Diabetes Mellitus	30 mg
Gymnema Sylvestre (Retz.) R.Br. ex Sm.-Meshashringi, Gurmur–Cowplant [24]	Anti-diabetic	30 mg
Pterocarpus Marsupium Roxb. P. Indicus-Pitasara – Indian kino tree [21]	Anti-diabetic	20 mg
Glycyrrhiza Glabra Linn-Mulethi (Yashtimadhu) – Licorice [21]	Nerve Tonic, Diabetes Mellitus, Liver and bladder disease conditions	20 mg
Syzygium Cumini (L.) Skeels-Jamun (Jambu) – Indian Blackberry [25]	Anti-diabetic	20 mg
Asparagus Racemosus Willd.-Shatavari – Buttermilk root, Wild asparagus [26]	Anti-diabetic	20 mg
Boerhavia Diffusa Linn-Punarnava – Red hogweed [21]	Hepatoprotective	20 mg
Sphaeranthus Indicus Linn-Mundatika – East Indian globe thistle [21]	Liver, skin, blood sugar and respiratory conditions.	10 mg
Tinospora Cordifolia (Willd.) Miers.-Guduchi – Heart-Leaved Moonseed [21]	Nerve tonic, Anti-diabetic	10 mg
Swertia Chirata Buch.-Ham-Kairata – Chitretta [21]	Anti-diabetic, Hepatoprotective.	10 mg
Tribulus Terrestris Linn-Gokshura – Land-Caltrops, Puncture Vine [21]	Anti-diabetic	10 mg
Phyllanthus Amarus Schumach. & Thonn-Bhumyaamlaki – Carry me seed [22]	Anti-diabetic	10 mg
Gmelina Arborea Roxb.-Gumbhari – Candahar tree, White Teak [21]	Anti-diabetic	10 mg
Gossypium Herbaceum Linn.-Karpasi – Cotton plant seed [21]	Nerve tonic	10 mg
Berberis Aristata DC.-Daruhaldi (Daruharidra) – Indian berberry [25]	Anti-diabetic	5 mg
Aloe Barbadensis Mill.-Guarpatha-Indian Aloe, Aloe Vera [21]	Anti-aging, Detoxification, Anti-diabetic	5 mg
Triphala [21]	Detoxifier	3 mg
Momordica Charantia Linn-Sushavi – Bitter gourd [25]	Detoxifier	20 mg
Piper Nigrum Linn-Kali Marich – Black Pepper [27]	Anti-diabetic	10 mg
Ocimum Sanctum Linn-Tulsi – Holy Basil [24]	Anti-diabetic	10 mg
Abutilon Indicum Linn-Atibala-Indian Mallow [21]	Anti-diabetic	10 mg
Abhrak, Vignadi, Yashad bhasma [22]	Anti-diabetic	10 mg
Praval bhasma [22]	Dyspepsia	10 mg
Rumex Maritimus Linn-Jungli palak – Golden dock [21]	Nerve tonic	5 mg
Vanga Bhasma (Bang Bhasma) [22]	Dyspepsia, urinary disorders	5 mg
Curcuma Longa Linn-Haldi-Turmeric [21]	Detoxifier	10 mg
Purified Shingraf-Cinnabar, Quick Silver [22]	Heart diseases	5 mg
Trikatu [22]	Heart diseases, Anti-diabetic	5 mg



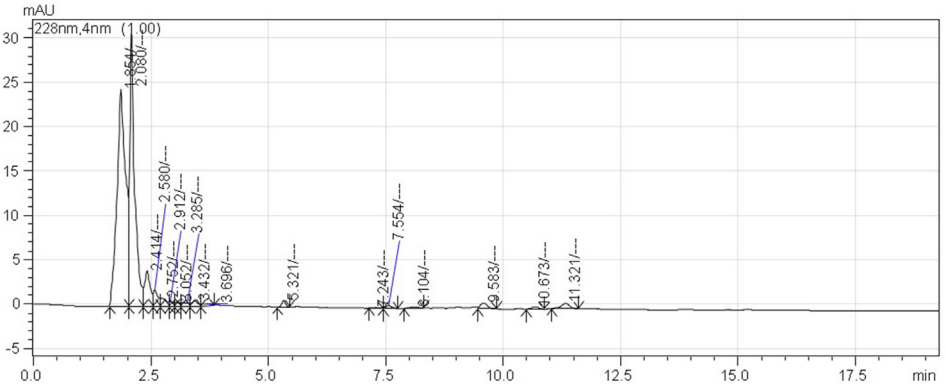
A

Blank rat plasma



B

Blank rat plasma spiked with working solution of glimepiride at the concentration of 25 ng/ml



C

Diabecon formulation

Fig. 1. Chromatograms A blank rat plasma, B blank rat plasma spiked with working solution of glimepiride at the concentration of 25 ng/ml, C diabecon formulation.

Table 2
Recoveries, intra-day and inter-day precision and accuracy of GP in rat plasma QC samples.

Concentration (ng/ml) of GP	Intraday precision[a]			Interday Precision[a]			Recovery (%)[a]		
	Estimated conc. (ng/ml)	Precision[a] (%CV)	Accuracy[a] (%RE)	Estimated conc. (ng/ml)	Precision[a] (%CV)	Accuracy[a] (%RE)	Mean	SD	%CV
75	68.61	0.42	−8.52	68.74	0.21	−8.34	91.48	0.38	0.42
500	463.21	0.32	−7.36	451.80	0.39	−9.64	84.66	1.02	1.21
800	789.14	0.33	−1.36	785.12	0.26	−1.86	99.08	0.63	0.63

[a] Mean of six determinations.

used to prepare distilled water (Millipore, Sigma). Collected water sonicated and filtered (0.45 μm) to remove air bubbles. Nicotinamide and Streptozotocin was acquired from Sigma Aldrich, Mumbai, India. All other reagents used were of AR grade.

2.2. Animals

Male Wistar rats weighing about 205 ± 245 gm, aged 6–7 weeks were used in the current investigation (Crystal Biological Solutions, Pune, India). The animals were maintained at the animal house facility of the institute. The animals were accommodated in polypropylene cages with solid bottoms and bedding made of autoclaved clean rice husk. The temperature was kept at 22–25 °C with a humidity of 60 ± 10% and a cycle of 12 h of light/darkness with a minimum of 15 air changes per hour. One week earlier in the beginning of this investigation, all animals were habituated to laboratory conditions. The animals were fasted overnight before the investigation, but were given water *ad libitum*. All the processes were carried out as per the recommendations of the "Committee for the Purpose of Control and Supervision of Experiments on Animals" New Delhi. The ethical clerance was obtained before the start of the study from the institutional animal ethics committee (DYPIPSR/IAEC/May17–18/P-24, CPCSEA Reg. No.: 198/PO/Re/S/2000/CPCSEA).

2.2.1. Induction of diabetes

To induce diabetes, 1 ml/kg b.w. intraperitoneal injections of STZ (55 mg/kg b.w.) in 0.1 M citrate buffer (pH 4.5) and Nicotinamide (110 mg/kg b.w.) in normal physiological saline were prepared. Nicotinamide (110 mg/kg, i.p.) was initially administered to overnight fasted Male Wistar rats. After 20 min of administration of nicotinamide, streptozotocin was injected i.p. to each rat. After 3 days, the animals were anesthetized with a ketamine and xylazine (1:1) i.p. Dose was calculated as per the body weights of the animals. The glucose level was measured using a commercially available glucometer (Accu Check, Roche Diabetes Care Pvt. Ltd, India). For further research, diabetic rats with a FBGL (Fasting Blood Glucose Level) of 250 mg/dL or more was chosen and divided into different groups.

2.3. PK and PD experimental design

The 21 days *in vivo* study was planned to investigate the effects of DB on the PK and PD of GP in diabetes induced (Nicotinamide-STZ) male Wistar rats. The diabetic rats were divided into 5 groups of six animals (n = 6) each at random.

Group I Control (C), administered with distilled water (Vehicle) (1 ml/kg, p.o.) daily.
Group II Diabetic control (DC), diabetes induced (Nicotinamide-STZ) male Wistar rats.
Group III (GP) diabetic rats were gavaged with GP at the dose of 0.82 mg/kg, p.o.
Group IV (DB) diabetic rats were gavaged with DB at dose 110.95 mg/kg, p.o.
Group V (GP + DB) diabetic rats co-administered with GP (0.82 mg/kg p.o.) and DB (110.95 mg/kg p.o.).

The freshly prepared solutions of GP and DB in 5% CMC in distilled water were delivered orally using an oral gavage needle. DB was given first to Group V animals, followed by GP. On the basis of the body surface area of rats, dosages of GP and DB were calculated to their respective animal effective doses by considering human doses and using the relevant conversion factors.

2.3.1. Sample preparation

The liquid–liquid extraction (LLE) technique was used to process the samples. A volume of 0.4–0.5 ml of blood was drawn from each rat via the retro-orbital plexus into eppendorf tubes containing 50 μL of 0.5 M EDTA and centrifuged at 4000 rpm for 20 min to obtain plasma. In polypropylene centrifuge tubes (1.5 ml), 100 μL plasma, 100 μL aliquots of the GP working standard solution, and 100 μL aliquots of IS solution were added. The tubes were shaken for 5 min, and then 100 μL of methanol was added as a precipitating solvent and vortexed for 5 min. The tubes were then further centrifuged at 3000 rpm for 20 min. The clear supernatant phase obtained was separated and evaporated in a vacuum oven and reconstituted with 1 ml of the mobile phase; 20 μL of each sample was injected into the HPLC system.

Table 3
Stability of Glimepiride in Rat Plasma and other validation parameters under Different Conditions.

Validation Parameter	Nominal Conc. (ng/ml)[a]	Determined conc. (ng/ml)[a]	%CV[a]
Short term stability	75	74.70	7.16
	800	781.88	5.20
Freeze thaw stability	75	76.83	8.82
	800	790.67	6.16
Long term stability	75	77.17	7.75
	800	794.85	5.96
Incurred sample reanalysis	75	79.41447672	8.86
	800	808.01	6.5
Matrix effect	–	Nil	–
Dilution integrity	75	80.92527574	9.16
	800	821.30	4.03

[a] Mean of six determinations.

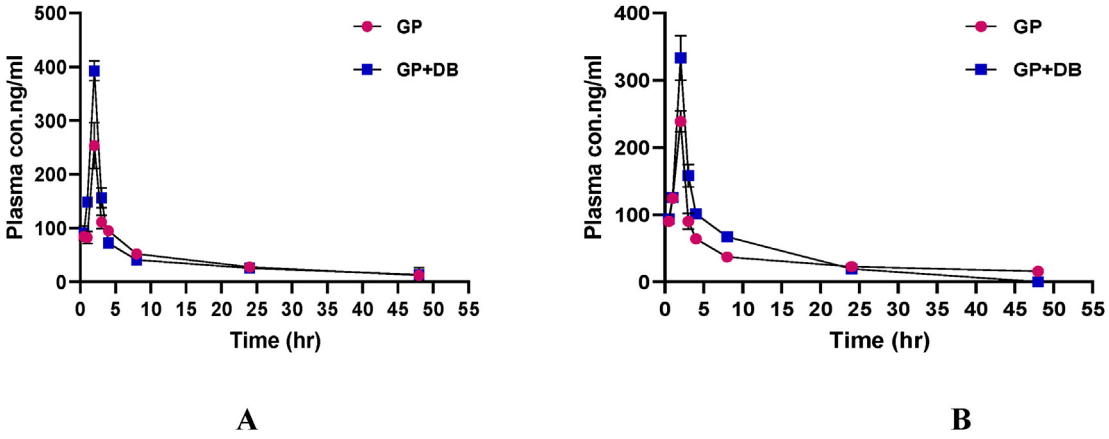


Fig. 2. Plasma concentration of glimepiride VS time profile glimepiride (0.82 mg/kg) and [glimepiride (0.82 mg/kg) + diabecon (110.95 mg/kg)] groups respectively. Plasma concentration Values are expressed as mean ± SEM. **A**: On 1st day, **B**: After 21 days.

2.4. In vivo pharmacokinetic study

After the completion of the treatment period, on the 21st day, blood was collected from the retro-orbital plexus (0.4–0.5 ml) of each animal which were previously fasted for 16 hrs but provided with water *ad libitum*, in eppendorf tubes containing EDTA. Blood was collected from groups III to V at time intervals of 0, 0.5,1, 2, 3, 4, 6, 8, 24 and 48 hrs on day 1 and day 21 of the study. Plasma was separated and stored at −20 °C until it was analyzed and PK parameters were estimated.

2.5. Instrumentation and initial chromatographic conditions

The Shimadzu (LC20AD) HPLC system composed of a quaternary pump, a manual 20 μL fixed loop rheodyne injector with a photodiode array (PDA) detector was used for the chromatographic analysis. LC solution software was used for processing of data. The chromatographic separation was performed using Kromasil 100 C_{18} analytical column (250mm × 4.6 mm, 5 μm). During the preliminary investigations, the chromatographic separation of GP and IS were carried out using different columns, different mobile phases, changing flow rate and using gradient systems to avoid interference of the various peaks of DB formulation. Initially ACN: 10 mM phosphate buffer, ratio 80:20 was used which resulted in peak distortion with tailing factor >2. Changing the ratio and pH of the solvent system modified the RT but with a low number of theoretical plates (N < 2000). Also the strength of the buffer was varied to get good separation between GP and IS peaks (Rs < 2). A mixture of ACN and 30 mM phosphate buffer pH 4, however, gave satisfactory results (T = 1.09, N = 17,346, Rs > 2 between adjacent peaks) and satisfied the system suitability criteria. After several trials, isocratic system [(Acetonitrile: 0.03 M potassium dihydrogen phosphate pH-4 adjusted with 1% orthophosphoric acid (55:45 v/v)] which gave sharp and resolved peaks with minimal tailing of GP & gliclazide (IS) and without any interference of the multiple peaks observed for DB formulation was selected as the optimized chromatographic conditions for analysis. Flow rate was maintained at 1 ml/min. At 228 nm, the chromatographic separation was measured. Standard stock solutions of GP (100 mg/ml) and IS (10 mg/ml) were prepared separately in the mobile phase and further diluted to prepare the working standard solutions.

The developed method was validated as per USFDA guidelines 2018. Lower Quality Control (LQC): 75 ng/ml, Middle Quality Control (MQC): 500 ng/ml, and Higher Quality Control (HQC): 800 ng/ml were used as the Quality Control standards (QC).

2.6. Pharmacodynamic (PD) study

On the 21st day of the study, the separated plasma samples of the Wistar rats of each group were evaluated for estimation of the PD and other biochemical parameters.

2.6.1. Estimation of PD parameters

Fasting Blood Glucose levels (FBGL), change in body weight, HbA1c and biochemical parameters i.e. total cholesterol (TC), triglyceride (TG) were measured using Pathozyme diagnostic kits.

2.6.2. Histopathology of rat pancreas

After accomplishment of the investigation, the rats were sacrificed and the pancreas were dissected and cleaned with cold saline solution. Then using 10% formaldehyde, the tissues were fixed. The tissue histopathology was carried out at Crystal Biologicals

Table 4
Pharmacokinetic parameters of glimepiride in diabetic rats treated with glimepiride alone and glimepiride co-administered with diabecon.

Pharmacokinetic parameters	Day 1		Day 21	
	GP alone group	GP + DB group	GP alone group	GP + DB group
C_{max} (ng/mL)	253.54 ± 4.25	392.57 ± 4.87*	238 ± 3.45	333.5 ± 2.50***
T_{max} (h)	2	2	2	2
AUC $_{(0-48\ hrs)}$ ng*hr/ml	4193.73 ± 14.48	4396.629144 ± 13.25**	3657.45 ± 15.40	4613.98 ± 13.78***
Elimination rate constant (k_e) hr-1	0.047529885 ± 1.15	0.053688873 ± 0.95***	0.04527543 ± 0.05	0.054502385 ± 0.03
Elimination half-life ($t_{1/2}$) hr	14.58030024 ± 0.59	12.90770251 ± 0.89**	15.30 ± 0.54	12.71 ± 0.48***
Volume of distribution (V_d) L	70.48790304 ± 2.89	59.30615121 ± 2.14****	77.72 ± 3.02	56 ± 1.37***
Clearance of drug (CL)L/hr	3.350281955 ± 1.48	3.184080417 ± 1.47*	3.5 ± 1.41	3.06 ± 1.23***

*Data expressed as mean ± SD. P-value <0.05 is considered as significant; *p < 0.05, **p < 0.01, ***p < 0.001 compared to the glimepiride alone treated group.

pathology laboratory, Pune, India. The preserved pancreas from all the groups were processed routinely and embedded in paraffin. The pancreas sections (3-5μ thickness) were stained with hematoxylin and eosin and histopathological changes were examined under a light microscope.

2.7. Statistical analysis

Individual rat plasma concentrations versus time profiles were computed using WinNonlin software version 5.2. (Pharsight Corporation, Mountain View, CA, USA). The observed individual GP plasma concentration−time data were used to calculate the peak plasma concentration (C_{max}) and the time to achieve C_{max} (t_{max}). The PK parameters assessed were area under the curve (AUC_{0-48hr}), volume of distribution (V_d), elimination rate constant (k_e), elimination half-life $t_{1/2,}$ CL. The maximum plasma GP concentration (C_{max}) and the time to reach C_{max} (T_{max}) were acquired from the plasma concentration−time curve. Statistical analysis was carried out using one/two way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using InStat 3 software (GraphPad Software, CA, USA). The differences were considered to be significant at $P < 0.05$.

3. Results

3.1. RP-HPLC/PDA bio analytical method validation for HDI study

As observed in Fig. 1, no interference peaks from endogenous compounds were found at the RT of (GP) 13.834 ± 0.5 min and (IS) 8.990 ± 0.3 min, thereby confirming the specificity of the method. The calibration curve of GP (25−1000 ng/ml) was linear with correlation coefficient $r^2 = 0.9954$, LLOQ of 25 ng/ml. In the intraday and interday precision studies, (% CV) values for GP were calculated and are summarized in Table 2 (<15% at all QC concentrations). The accuracy (% R.E) for GP was within the range of −1.0 to −10%. After several trials, methanol was selected as the solvent to extract GP and IS from plasma. As indicated in Table 2, the plasma extraction recovery at all QC concentration for GP was between 80 and 100% (% CV<15%) and 90 ± 5% for Gliclazide (IS). The stability analysis of GP in plasma is summarized in Table 3. GP was found to be stable in rat plasma for 3 hrs at room temperature, and for a minimum of 30 days when stored at −20 °C with a % CV <15% at LQC and HQC. The % CV of QC samples was <15% between initial concentrations and concentrations obtained after three freeze−thaw cycles. GP was also stable in the LLE solvent (methanol) for 24 hrs at 4°C.The precision and accuracy of the sample reanalysis of PK study samples of GP were found to be within acceptable limits. In the present method, no significant matrix interference was observed at the RT of GP and IS peak. Dilution integrity was found to be within 15% of nominal concentration and 15% CV, as summarized in Table 3.

3.2. In vivo PK studies of GP when co-administered with herbal formulation DB in diabetes induced (Nicotinamide-STZ) rats

The developed RP-HPLC/PDA method was effectively used to analyze the PK parameters of GP on concomitant administration with DB. In diabetes induced (Nicotinamide-STZ) Wistar rats, the pharmacokinetic HDI on concomitant administration of GP at a dose (0.82 mg/kg p.o.) with herbal formulation DB at a dose (110.95 mg/kg p.o.) was investigated for 21 days. Fig. 2 illustrates the concentration in plasma versus time profiles of GP after oral administration of DB on day 1 and day 21. Table 4 summarizes the pharmacokinetic parameters. According to the pharmacokinetic data generated; the co-administration of DB and GP resulted in C_{max} of 238 ± 3.45 ng/ml for GP at the T_{max} 2 hr of the study. The Vd

Table 5
Values of FBGL, Change in weight, in rats treated with GL, DB, GL + DB for 21 days.

Days	Normal		DC		GP		DB		GP + DB	
	FBGL	Body Wt.	FBGL	Body Wt.	FBGL	Body Wt.	FBGL	Body Wt.	FBGL	Body Wt.
1	101.67 ± 5.16	230 ± 3.30	331.33 ± 14.63	244.16 ± 3.51	302.5 ± 5.12	227.7 ± 5.81	311.17 ± 9.46	224.5 ± 2.26	304.66 ± 8.86	233.66 ± 4.49
7	101.33 ± 3.95	244.16 ± 3.51	362.33 ± 10.43	181.66 ± 2.60	271.6 ± 62.40	156.5 ± 3.60	270.33 ± 9.01	171.33 ± 3.46	268.17 ± 4.08	180.83 ± 1.72
14	102.16 ± 4.30	257.17 ± 3.39	380.83 ± 11.24	133.5 ± 3.81	225.5 ± 8.94	174.16 ± 3.60	226.83 ± 8.54	184.33 ± 1.94	225.67 ± 4.44	194 ± 1.82
21	102.33 ± 4.07	269.83 ± 3.10	401.83 ± 10.69***	98.5 ± 3.15***	181.5±±14.71###	195 ± 4.20###	174.83 ± 7.41###	201 ± 1.69###	178.83 ± 2.99###	205.66 ± 1.47###

Data expressed as mean ± SEM, * Significant with respect to NC, # Significant with respect to DC ($p < 0.001$).

value was estimated to be 77.72 3.02 L/kg with CL of 3.5 ± 1.41 L/h and a $t_{1/2}$ of 15.30 ± 0.54 h. Co-administration of DB herbal formulation in Nicotinamide-STZ induced rats resulted in an increase in C_{max} of GP from 238 ± 3.45 ng/ml to 333.5 ± 2.50 ng/ml. However no shift in T_{max} was observed (Fig. 2). Consequently, a slight decrease in V_d (56 ± 1.37 L/kg), CL (3.06 ± 1.23 L) and $t_{1/2}$ (12.71 ± 0.48 h) was observed. Pharmacokinetic profile also showed a slight increase in AUC_{0-48hr} (3657.45 ± 15.40 h*ng/ml to 4613.98 ± 13.78 h*ng/ml) in the presence of DB.

3.3. Pharmacodynamic interactions of DB and GP in diabetes induced (Nicotinamide-STZ) rats

3.3.1. Effect of GP, DB and GP + DB on FBGL

In the PD studies, the FBGL in diabetic control group was significantly increased ($p < 0.001$) in comparison to control group (Table 5). In comparison to diabetic control group with fasting blood glucose 401.833 ± 10.69 mg/dL, oral administration of GP, DB, and GP + DB at corresponding doses for 21 days resulted in a significant drop in FBGL ($p < 0.001$) to 181.5 ± 14.71, 174.84 ± 7.41, and 178.83 ± 2.99 mg/dL respectively. When comparing the DB and GP+DB groups to the GP alone group, it can be noted that the reduction in FBGL was more significant ($p < 0.001$).

3.3.2. Effect of GP, DB GP + DB on the body weight of animals

This investigation employed animals weighing between 205 ± 245 gm. During the investigation, it was found that the body weight of rats of the control group gained consistently during the 21 days of study, whereas the body weight of the Nicotinamide-STZ-induced diabetes control group was significantly ($p < 0.001$) lowered than the control group. From the 7th day after receiving GP, DB, or GP+DB, a significant ($p < 0.001$) gain in body weight was seen when compared to the diabetic control group (Table 5). After 21 days of continuous treatment of GP, DB, and GP+DB, the body weight of the rats was nearly restored to its initial level.

3.3.3. Effect of GP, DB and GP + DB on TG and TC levels

On comparison of the diabetic control group to the control group, plasma TC and TG levels were significantly greater ($p < 0.001$) in the diabetic control group. After 21 days of treatment, diabetic rats treated with DB exhibited lower plasma TC and TG levels (121.33 ± 6.56 mg/dL and 103.20 ± 5.5 mg/dL, respectively) than diabetic control rats (152 ± 3.73 mg/dL and 161.85 ± 6.09 mg/dL, respectively). In comparison to the diabetic control group, GP lowered plasma TC and TG levels to 107.5 ± 4.54 mg/dL and 114.19 ± 4.96 mg/dL, respectively. In comparison to the diabetic control group, GP+DB treated group resulted in a significant decrease ($p < 0.001$) in plasma TC and TG levels (108 ± 5.24 mg/dL and 97.71 ± 5.41 mg/dL, respectively). The current investigation revealed that the diabetes induced (Nicotinamide-STZ) rats demonstrated significantly higher levels of plasma TC and TG ($p < 0.001$). On the other hand, the GP+DB combination treatment significantly lowered TC and TG levels ($p < 0.001$) (Table 6).

3.3.4. Effect of GP, DB and GP + DB on glycosylated haemoglobin (HbA1c)

The increase in blood sugar levels in diabetic condition was confirmed by measuring glycosylated haemoglobin. In comparison to the control group, diabetes induced (Nicotinamide-STZ) rats showed a significant increase ($p < 0.001$) in HbA1c. After 21 days of administration, the HbA1c level in the GP treatment group was lower (4.89%) than in the diabetic control group (8.79%). In comparison to the diabetic control group, DB resulted in an HbA1c level of 5.23%. The lowered levels of HbA1c (4.12%, $p < 0.001$) were also maintained during the treatment with GP+DB combination (Table 6).

3.3.5. Effect of GP, DB, and GP + DB on histopathological alterations of pancreas

Pancreatic histopathological changes are shown in Fig. 3. The extent of tissue necrosis was used to grade clinical manifestations. The histopathological examination of pancreas in control group showed no change (Grade 0) in islets of Langerhans, on the contrary in diabetic control group degenerative changes with infiltration of pancreatic cells and reduced dimensions of islets of Langerhans (Grade 2 − Animal showed infiltration of pancreatic cells.) were seen, **GP** group showed changes in islets of Langerhans in pancreas (Grade 0−Normal Pancreatic Cells) after treatment, **DB** exhibited marked changes with normal pancreatic cells and no any cellular infiltration (Grade 0) as compared to DC, **GP+DB** showed improvement and normal structure of islets (Grade 0), as illustrated in Fig. 3. The study indicated that the damage to the pancreatic tissue was found to be recovered in the GP and DB treated group. The GP + DB co-administered group also showed noteworthy decrease in necrosis to pancreas and revival of islet cells as compared with diabetic control rat group after 21 days of therapy as shown in the microphotographs.

4. Discussion

Patients suffering from diabetes frequently use oral hypoglycemic drug, along with commercially available herbal formulations [17]. As a result, when herbal formulations are co-administered with oral hypoglycemic medicines, an evidence-based assessment of risk against benefit is required. The focus of the current study was to estimate the *in-vivo* pharmacokinetic and pharmacodynamic interactions of most commonly used herbal formulation DB with GP, an antidiabetic agent, in diabetes mellitus induced (Nicotinamide-STZ) rats. The pharmacokinetic data attained in the investigation demonstrated that the co-administration of DB herbal formulation with GP in Nicotinamide-STZ induced rats resulted in an increase in C_{max} of GP. However no shift in T_{max} was observed. Consequently, a slight decrease in V_d, CL and $t_{1/2}$ was observed. Pharmacokinetic profile also demonstrated a slight increase in AUC_{0-48hr} in the presence of DB. In diabetic rats, the elevated plasma glucose level was significantly ($p < 0.001$) reduced by co-administration of GP+DB over 21 days as compared to the diabetic control group. Glimepiride increases peripheral glucose absorption by stimulating insulin granule production and improving insulin sensitivity in peripheral tissues, lowering plasma blood

Table 6
Values of FBGL, Change in weight, TC, TG in rats treated with GL, DB, GL + DB for 21 days.

Parameters	Normal	DC	GP	DB	GP + DB
TC (mg/dL)	46.16 ± 4.12	152 ± 3.73***	107.5 ± 4.54###	121.33 ± 6.56##	108.16 ± 5.24###
TG (mg/dL)	56.75 ± 5.06	161 ± 6.09***	114.19 ± 4.96###	103.20 ± 5.50###	97.71 ± 5.41###
HbA1c (%)	3.9	8.79***	4.89##	5.23#	4.12##

Data expressed as mean ± SEM, * Significant with respect to NC,# Significant with respect to DC ($p < 0.001$).

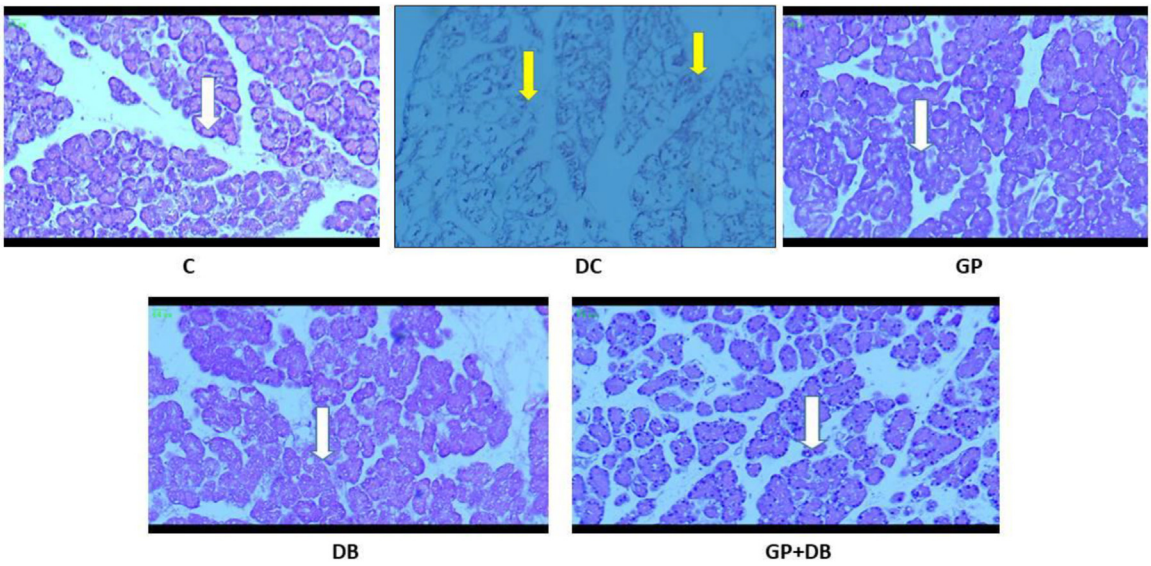


Fig. 3. Effect of Glimepiride (GP), Diabecon (DB) and Glimepiride + Diabecon (GP + DB) administration on histopathological change of rat pancreas. White Arrow – Normal Pancreatic Cells, Yellow Arrow – Cellular infiltration.

glucose and glycated haemoglobin (HbA1c) levels [37]. Diabecon, a polyherbal formulation, which comprises *Gymnema sylvestre, Eugenia jambolana, Glycyrrhiza glabra, Momordica charantia* as the key constituents present in it, and has been reported for its anti-diabetic and hypolipidemic activity [7].The decreased glucose level in the PD study suggested that co-administration of DB formulation and GP has a synergistic effect, possibly leading to increased intracellular insulin receptor activity, stimulation of pancreatic beta cells to release insulin, decreased hepatic glucose production, and improved pancreatic beta cell restoration. In addition, there was no incidence of hypoglycemia in the GP+DB treatment group. Dyslipidemia is recognized to be connected to diabetes. Higher TC and TG levels are signs of diabetic dyslipidemia. It was observed that GP+DB combination treatment reduced TC and TG levels more significantly ($p < 0.001$) with restoration of the body weight of the rats. HbA1c is one of the most reliable indicators of glycemic control in people with diabetes. Increased glycosylation of haemoglobin is a characteristic of diabetes (HbA1c). The effectiveness of the diabetes treatment is demonstrated by a significant drop in HbA1c values. The co-administration of GP+DB significantly lowered HbA1c levels in the current investigation when compared to the individual treatment groups, indicating strong pharmacodynamic interactions on co-administration of GP and DB. On the basis of histopathology studies, the diabetic control pancreas revealed pathological alterations. The treated groups, on the other hand, restored normal pancreatic cells and found no cellular invasion. However, furthermore clinical trials or case study in human volunteers are required to substantiate the findings of this preclinical experiments.

5. Conclusion

The combination therapy of Glimepiride and Diabecon did not show any adverse HDI in the pre-clinical experimentation in diabetic wistar rats and is safe to be investigated in a clinical set up. When compared to GP monotherapy, this combination therapy may be beneficial and prove to be useful to reduce the possibility of occurrence of comorbidities associated with diabetes mellitus. However, to substantiate the results obtained in pre-clinical studies, further research through randomized clinical trials in humans, is needed.

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Conflict of Interest

The authors declare there are no conflicts of interest.

Author contributions

Archana K. Thikekar: Methodology, Investigation, Formal analysis, Writing - Original Draft. **Asha B. Thomas:** Conceptualization, Writing - Review & Editing. **Sohan S. Chitlange:** Supervision. **Bhalchim Vrushali:** Investigation.

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