Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Pharmacokinetic and Pharmacodynamic Interactions of *Tinospora cordifolia* Aqueous Extract and Hypoglycemic Drugs in Streptozotocin-Induced Diabetes in Rats

Amisha Vora, Alice Varghese, Yusuf Kachwala, Ankit P Laddha[1], Manju Bhaskar[1], Jamal Akhtar[2], Pawan Yadav[2]

Departments of Pharmaceutical Chemistry and [1]Pharmacology, Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management, SVKM's NMIMS, Mumbai, Maharashtra, [2]Central Council for Research in Unani Medicine, Ministry of AYUSH, Government of India, New Delhi, India

Submitted: 25-06-2019 Revised: 06-09-2019 Published: 31-03-2020

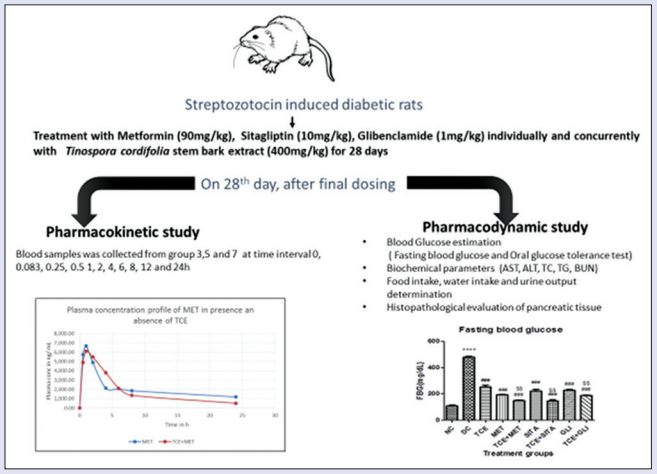
ABSTRACT

Context: *Tinospora cordifolia* (Willd.) Miers is an antidiabetic herb globally marketed as a single or polyherbal formulation. The pharmacological activities of *T. cordifolia* suggest its potential to be co-administered with commonly prescribed oral hypoglycemic drugs in the therapeutic management of diabetes mellitus. **Aims:** The aim of this study was to evaluate the potential pharmacokinetic (PK) and pharmacodynamic (PD) interactions of the aqueous extract of *T. cordifolia* (TCE) with metformin (MET), sitagliptin (SITA), and glibenclamide (GLI) in streptozotocin (STZ)-induced diabetes in rats. **Settings and Design:** The aqueous TCE (400 mg/kg, p.o.) was co-administered with MET (90 mg/kg, p. o.), SITA (10 mg/kg, p. o.), and GLI (1 mg/kg, p. o.) for 28 days in STZ-induced diabetes in rats. **Materials and Methods:** The PK parameters of the three drugs were calculated using Phoenix WinNonlin®, and PD activity was determined by estimating fasting blood glucose, liver and kidney function tests, and lipid parameters. Further, histopathological examination of pancreatic cells and estimation of pancreatic antioxidant enzymes was carried out. **Statistical Analysis Used:** The pharmacokinetic parameters were estimated using pharmacokinetic program WinNonlin® version 3.0 (Pharmasight corporation, Mountain view, CA). The pharmacodynamic parameters were analysed using Graph Pad prism Version 6.00 applying one-way ANOVA followed by the Tukey–Kramer post test. **Results:** No significant PK interaction was observed between TCE and the three oral hypoglycemics. However, a significant improvement was observed in glycemic control and the conditions associated with diabetes mellitus. In addition, no incidences of hypoglycemia were observed. **Conclusion:** The co-administration of TCE with MET, SITA, and GLI helped in better management of diabetes and associated comorbidities than their individual administrations. There were no significant PK interactions observed. Our findings provide insights into the safety and efficacy of the combination therapy of TCE with MET, SITA, and GLI in the management of diabetes and associated conditions.

Key words: Glibenclamide, interaction, metformin, pharmacodynamics pharmacokinetics, sitagliptin, *Tinospora cordifolia*

SUMMARY

- The main aim of this current study is to evaluate the potential pharmacokinetic (PK) and pharmacodynamic (PD) interactions of the aqueous extract of *Tinospora cordifolia* (TCE) with metformin (MET), sitagliptin (SITA), and glibenclamide (GLI) in streptozotocin (STZ)-induced diabetes in rats. The PK parameters of the three drugs were calculated using Phoenix WinNonlin®, and PD activity was determined by estimating fasting blood glucose, liver and kidney function tests, and lipid parameters. No significant PK interaction was observed between TCE and the three oral hypoglycemics. However, a significant improvement was observed in glycemic control and the conditions associated with diabetes mellitus. In addition, no incidences of hypoglycemia were observed. There were no significant PK interactions observed. Our findings provide insights into the safety and efficacy of the combination therapy of TCE with MET, SITA, and GLI in the management of diabetes and associated conditions.



Abbreviations used: MET: Metformin; SITA: Sitagliptin; GLI: Glibenclamide; STZ: Streptozotocin; TC: *Tinospora cordifolia*; DM: Diabetes mellitus; PPG: Postprandial plasma glucose; DPP-4: Dipeptidyl peptidase-4; CAM: Complementary and alternative medicine; T1D: Type 1 diabetes; PK: Pharmacokinetic; PD: Pharmacodynamic; STZ: Streptozotocin; TPC: Total phenolic content; TFC: Total flavonoid content; SD: Sprague Dawley; IAEC: Institutional Animal Ethics Committee; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; DC: Diabetic control; BSA: Body surface area; LC-MS: Liquid chromatography–mass spectrometry; PRM: Pramipexole; ESI: Electrospray ionization source; CE: Collision energy; CAD: Collision-activated dissociation; FBG: Fasting blood glucose; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; BUN: Blood urea nitrogen.

Correspondence:

Dr. Amisha Vora,
Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management,
SVKM's NMIMS, Vile Parle (West),
Mumbai - 400 056, Maharashtra, India.
E-mail: amisha.vora@nmims.edu

DOI: 10.4103/pm.pm_272_19

Access this article online
Website: www.phcog.com
Quick Response Code:

Cite this article as: Vora A, Varghese A, Kachwala Y, Laddha AP, Bhaskar M, Akhtar J, *et al*. Pharmacokinetic and pharmacodynamic interactions of *Tinospora cordifolia* aqueous extract and hypoglycemic drugs in streptozotocin-induced diabetes in rats. Phcog Mag 2020;16:S47-56.

INTRODUCTION

As an ayurvedic medicine, *Tinospora cordifolia* (Willd.) Miers (common name: Guduchi, family: Menispermaceae) (TC) has been found to be beneficial in treating diabetes mellitus (DM).[1] It is popularly used among the Indian tribal population in the treatment of diabetes, fever, jaundice, rheumatism, and urinary tract infections.[2] The Materia Medica and the Ayurvedic Pharmacopoeia of India describes the use of this herb as a potential antidiabetic agent. It is marketed globally as a monotherapy unit or as a constituent of polyherbal formulations.[3,4] A variety of phytoconstituents such as alkaloids, glycosides, steroids, tannins, saponins, diterpenoid lactones, and flavonoids have been isolated from the plant and their structures have been elucidated.[4-8] Many of the following pharmacological activities such as antihyperglycemic,[9,10] antihyperlipidemic,[11] antimicrobial,[12] and antioxidant activities[13] have been reported to be found in the different plant part extracts. Various studies performed on TC proposed different mechanisms in support of its hypoglycemic effects such as rejuvenation of islet cells of the pancreas, increased secretion of insulin, peripheral utilization of glucose,[14] and decreased absorption of glucose from the walls of the intestine,[15] and enhanced antioxidant activity by the liver cells.[16]

Metformin (MET), a biguanide agent, is used as a first-line therapy in treating type 2 diabetes. The drug assists in lowering the basal and postprandial plasma glucose levels in the blood. It does the work by reducing hepatic glucose production, intestinal absorption of glucose, and improved uptake of glucose and its utilization.[17,18] MET also acts on the insulin receptors and glucose transporters. There are few studies reporting the use of MET in type 1 diabetes (T1D). The US and UK guidelines have recommended considering MET as an add-on therapy to insulin in obese diabetic patients in order to improve glycemic control and to reduce the insulin requirement.[19]

Gliptins belong to a class of oral hypoglycemics which competitively antagonize dipeptidyl peptidase-4 (DPP-4) enzyme, thus leading to incretin hormones' prolonged action, which increases the synthesis of insulin and its release from beta pancreatic cells. Sitagliptin (SITA) is the first gliptin approved by the Food and Drug Administration (FDA) in 2006.[20] Various clinical studies have provided evidence for beneficial effects of SITA in T1D.[21] Similarly, glibenclamide (GLI), also known as glyburide, belongs to the sulfonylurea class of antidiabetic drugs. It is widely prescribed among diabetic population and acts by inhibiting ATP-dependent potassium efflux from pancreatic beta-cells, leading to calcium influx and activation of enzymes causing release of insulin.[22,23]

Complementary and alternative medicine is a widely used concept in the management of chronic conditions such as diabetes mellitus. T1D is highly prevalent worldwide, i.e., associated with various comorbidities such as reduced life expectancy and higher health-care costs.[24-27] Although insulin is given as a first-line treatment, oral hypoglycemics such as MET, SITA, and GLI are recommended as add-on therapies for reducing the insulin requirement.[20]

The antidiabetic herb *T. cordifolia* is also believed to lower blood glucose in diabetic patients. The patient might not be aware of the possible interactions while combining herb with any oral hypoglycemic drug. Hence, it is imperative to investigate the safety and efficacy of the combination. Our study aims at investigating the pharmacokinetic (PK) and pharmacodynamic (PD) interactions of the aqueous extract of *T. cordifolia* (TCE) with three of the commonly prescribed oral hypoglycemics in a rodent model with diabetes induced by streptozotocin (STZ).

MATERIALS AND METHODS

Drug chemicals and solvents

STZ was obtained from Sigma Chemical Co., USA. MET, SITA, and GLI were generous gifts from IPCA Laboratories, Mumbai, India. ERBA Diagnostic kits such as glucose oxidase peroxidase, aspartate aminotransferase, alanine aminotransferase, total cholesterol, triglyceride, and blood urea nitrogen were purchased from Noble Diagnostics, India. Methanol and acetonitrile of liquid chromatography–mass spectrometry (LC-MS) grade were obtained from Merck® India Ltd., Mumbai. Tert-butyl methyl ether, ethyl acetate, formic acid, and diethyl ether of analytical grade were procured from Qualigens Fine Chemicals, Mumbai, India. MET was obtained as a gift sample by U. S Vitamins, Mumbai, India.

Plant material

Fresh TC stem bark was obtained from Borivali National Park, Mumbai, India. The plant was identified and authenticated at Agharkar Research Institute, Pune, and the sample was deposited in their herbarium with a specimen number 15-236.

Extraction method

The double maceration method was used for the preparation of aqueous plant extract from the dried and powdered bark of *T. cordifolia*. A 7-day long maceration process was carried out that included dried bark powder of TC (500 g) with 5 L of distilled water. This was followed by filtration of the extract. The obtained filtrate was refrigerated till further use. Seven more days of maceration of marc was conducted with addition of fresh distilled water, following the same method as stated above, and the filtrate obtained was added up to the previously stored filtrate. The combined filtrate was concentrated by spray drying that afforded extract in the dried powder form.

Total phenolic content estimation

For this purpose, the Folin–Ciocalteu method was implemented using gallic acid for standard curve.[28] The total phenolics were expressed as milligrams of gallic acid equivalents/grams of the extract.

Total flavonoid content estimation

Total flavonoid content (TFC) of TCE was estimated by modified aluminum chloride colorimetric assay.[29] Flavonoid content was recorded as milligrams of quercetin equivalents/grams of the extract.

Standardization of *Tinospora cordifolia* aqueous extract

Quantification of berberine

Quantity of berberine in TCE was determined by high-performance liquid chromatography (HPLC) using a method described by Srinivasan *et al.*[30]

Experimental animals

The study included male Sprague Dawley (SD) rats which were around 8 weeks in age and weighed around 180–220 g. The SD rats were purchased from Bharat Serums and Vaccines Ltd., Mumbai, India, and housed in SVKM's animal facility prior to the experiment. The animals were housed in clean polypropylene cages under standard conditions of humidity (70±5%), temperature (25±2°C) and light (12 h light/12 h dark cycle). The diet included multigrain basal nutritional diet (Nutrimix Laboratory Animal Feed, Maharashtra, India) along with *ad libitum* access to purified water. The Institutional Animal Ethics Committee

approved this experimental protocol for the antidiabetic study, and it was conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India (Registration No. 1830/PO/Re/S/15/CPCSEA).

Pharmacokinetic interactions of TCE with metformin, sitagliptin, and glibenclamide in diabetic rats

Induction of diabetes

Except for the six animals of Group I (the normal control [NC] group), diabetes was induced in the rest of the animals using STZ (55 mg/kg, i. p.) [Table 1]. STZ was prepared freshly in citrate buffer (pH 4.4). The plasma blood glucose was measured 7 days after the administration of STZ. Animals with more than 250 mg/dL of blood glucose level were considered to be diabetic and selected for further study.

Grouping of animals

The selected rats with induced diabetes were randomly distributed in eight different groups (Group II to Group IX), each containing six rats. The nondiabetic rats were designated as Group I (the NC group) and were administered with 1 ml/day distilled water for 28 days. Group II was considered as diabetic control (DC), and the animals in this group were not given any treatment rather administered distilled water 1 ml/kg. Groups III, IV, V, VI, VII, VIII, and IX were administered with 400 mg/kg TCE, 90 mg/kg MET, 400 mg/kg TCE followed by 90 mg/kg MET, 10 mg/kg SITA, and 400 mg/kg TCE followed by 10 mg/kg SITA, 1 mg/kg GLI, and 400 mg/kg TCE followed by 1 mg/kg GLI respectively, via oral route using dosing needle. The solutions of MET, SITA, and TCE were prepared in water and GLI in 0.5% w/v carboxymethyl cellulose solution. In the combination treatment groups, the drug was administered first followed by TCE at an interval of 30 min. In order to convert the dosage from human value to rats, the body surface area normalization approach was applied.[31]

Pharmacokinetic study

For PK interaction studies, the Groups IV, V, VI, VII, VIII, and IX were further divided into two subgroups of A and B. On the 28th day, after the last dose of treatment, the rats were anesthetized with isoflurane. Approximately 0.25 ml of blood was withdrawn (except at 0 h when 0.4–0.5 ml blood was withdrawn) from retro-orbital plexus and collected in heparinized tubes at predetermined time intervals of 0, 0.5, 1, 2, 4, 6, 8, and 24 h. Blood for time points 0, 1, 4, and 6 h was withdrawn from subgroup A, whereas that for time points 0.5, 2, 8, and 24 h was withdrawn from subgroup B. The total blood withdrawn did not exceed 20% of the body blood volume. Centrifugation of blood samples was performed at 4000 g for 10 min at 4°C. The obtained plasma was stored at −80°C till further experimentation. The 0-h samples were also used for the PD parameters.

Bioanalytical method by liquid chromatography–mass spectrometry

We developed individual LC-MS methods for estimation of MET, SITA, and GLI in rat plasma. For MET quantification, GLI was used as an internal standard (IS), and for determination of GLI, MET was used as an IS. For the estimation of SITA, pramipexole (PRM) was used as an IS. These methods were developed on Shimadzu C8 (150 mm × 4.6 mm, 5 µm particle size) analytical column which was maintained at 40°C. The mobile phase consisted of 0.1% formic acid (A) and methanol (B). For SITA, an isocratic elution method comprising 60%B was used (total run time = 4 min), and for MET and GLI, a gradient method (time in min/%B – 0/10, 2/10, 4/95.12/95, 12.5/10, and 14/10) with a total run time of 14 min was used. The flow rate of the mobile phase under gradient condition was kept to 0.5 ml/min 10 µl injection volume. The mass spectrometer consisted of electrospray ionization source (ESI) and triple-quadrupole mass analyzer. The quantification of drugs and IS were conducted using multiple reaction monitoring (MRM) technique in positive ionization mode. The ion-source parameters were set at nebulizer gas flow of 3 L/min; drying gas flow rate at 15 L/min, ion spray voltage at 4500 V, heat block temperature at 400°C, and desolvation line temperature at 250°C and collision energy (CE) were set at −14, 23, −13 for MET, −20, −28, −26 for SITA, −16, −40, −29 for GLI, and −15, −28, −40 for PRM. At 230 kPa, nitrogen was implemented as collision activated dissociation (CAD) gas, whereas quadrupole 1 and quadrupole 3 (with 100 ms dwelling time) were set up at unit resolution. The 5.75 version of the Lab Solution software (LabSolutions, Shimadzu, Japan) was used for setting up the parameters and data analysis.

Stock and spiking solutions and partial validation of bioanalytical method

Primary stock solutions, each of MET, SITA, and GLI, were made at concentration of 1000 µg/ml. They were further diluted with methanol to obtain individual working standards in the range of 0.025–10 µg/ml using methanol. 10 µl of each working standard solution of MET, SITA, and GLI was spiked in 90 µl of blank rat plasma to afford final concentrations ranging from 2.5–1000 ng/ml. The quality control (QC) standards for each drug were prepared at 2.5 ng/ml (lower limit of quantitation QC), 100 ng/ml (low QC), 500 ng/ml (medium QC), and 10000 ng/ml (high QC). IS was added to each level of drug at the final concentration of 500 ng/ml. Ethyl acetate (1 ml) was added as the extraction solvent. The samples were vortexed for 10 min and centrifuged at 4000 g for 10 min. 900 µl of the supernatant was withdrawn, the solvent was evaporated using nitrogen evaporator, and the residue was reconstituted with 100 µL of mobile phase. 10 µl of the samples was injected into the HPLC/ESI tandem mass spectrometry (HPLC-ESI-MS/MS).

Bioanalysis

Blood samples of animals from each group (Group IV to Group IX) at various time points were processed in a similar way, and the developed method was applied to quantitate MET in blood samples of Groups IV

Table 1: Main pharmacokinetic parameters of metformin, metformin + *Tinospora cordifolia*, sitagliptin, sitagliptin + *Tinospora cordifolia*, glibenclamide, and glibenclamide + *Tinospora cordifolia* in streptozotocin induced diabetic rats (*n*=9)

PK parameters	MET	MET+TCE	SITA	SITA + TCE	GLB	GLB + TCE
C_{max} (ng/mL)	6768.127±866.838	6112.073±690.383	18,916.15±3837.346	17,246.27±4044.364	15,363.76±2036.1	21,935.16±3451.3
T_{max} (h)	0.833±0.167	1±0.5	2.85±0.75	2.063±0.76	5.333±0.65	7.333±0.36
AUC (0-24 h)	52,166.97±4080.145	42,571.41±2391.196	113,214.5±52,109.08	127,146.1±7533.4	256,768.9±20,124.33	313,976.1*±16,811.44
Elimination rate constant (Ke)	0.031,799	0.081,703	0.184,788	0.271,086	0.030	0.063

Data expressed as mean±SD. *Significant with respect to drug alone treated group. SD: Standard deviation; MET: Metformin; *T. cordifolia*: *Tinospora cordifolia*; TCE: *T. cordifolia*; SITA: Sitagliptin; GLI: Glibenclamide; AUC: Area under the curve; C_{max}: Concentration maximum; T_{max}: Time of maximum; PK: Pharmacokinetic

and V, SITA in Groups VI and VII, and GLI in Groups VIII and IX. PK program WinNonlin version 3.0 of Pharmasight Corporation (Mountain View, CA, USA) was used to determine the values of various PK parameters, including concentration maximum (C_{max}), area under the plasma concentration-time curve (AUC), and terminal elimination half-life ($t_{1/2}$). All the estimated values were denoted in mean ± standard error of mean (SEM), excluding the T_{max} which was mentioned as median.

Pharmacodynamic interactions in streptozotocin-induced diabetic rats

Blood samples collected at 0 h were subjected to biochemical tests such as fasting blood glucose (FBG) and liver function parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lipid profile parameters including total cholesterol (TC) and triglyceride (TG), and kidney parameters, namely blood urea nitrogen (BUN). They were estimated using ERBA Diagnostic kits as per manufacture's protocol. Food intake, water intake, and urine output were monitored using a metabolic cage.

Rats from each group were euthanized with their pancreas isolated for further analysis. The organs were washed in saline solution (cold), and a part of it was fixed using 10% formaldehyde aiming at its histopathological examination. H and E stain was used in the tissue section and observed through the light microscope for any form of histoarchitectural changes. The extent of necrosis of islet cells in the section determined their pathological grades.

Antioxidant activity in pancreatic tissue

The rest of the tissue was weighed and homogenized using homogenizer (REMI Electrotechnik Pvt. Ltd.) with 10 ml of 0.1 M Tris-HCl buffer (pH 7.4). Homogenate was then centrifuged at 4000 g for 10 min, and the supernatant was used for determination of protein content the Lowry method,[32] followed by the assay of the antioxidant enzymes glutathione peroxidase (GPx),[33] glutathione reductase by Dubler and Anderson method,[34] superoxide dismutase (SOD),[35] and catalase (CAT).[36] Thiobarbituric acid reactive substances (TBARS) were determined by a method described by Ohkawa *et al.*[37]

Statistical analysis

The final findings of this PD study were expressed in terms of mean ± SEM of six animals from each group. The statistical analysis was performed using GraphPad Prism version 6.00 of GraphPad Software Inc., San Diego, USA. The outcomes were studied through the one-way ANOVA and Tukey–Kramer posttest (significance at $P < 0.05$).

RESULTS

Total phenolic content

The total phenolic content of aqueous extract was found to be 0.155 ± 0.085 mg of gallic acid equivalents/g of the extract.

Total flavonoid content

TFC of aqueous extract was found to be 1.817 ± 0.056 mg of quercetin equivalent/g of the extract.

Standardization of TCE

The content of berberine in the TC aqueous extract was calculated as 0.37% w/w using the HPLC method.

Pharmacokinetic interactions of TCE with metformin, sitagliptin, and glibenclamide

Bioanalytical method

HPLC-ESI MS/MS method for individual estimation of MET, SITA, and GLI in rat plasma was developed. Their retention times were found to be 3.4, 0.642, and 10.12 min, respectively. The Retention time (RT) of PRM used as an IS for SITA was found to be 0.483 min [Figure 1]. Their masses were confirmed at m/z 130.0 for MET, 408.20 for SITA, 494.00 for GLI, and 212.20 for PRM. Further collision-induced fragmentation led to production of ions of m/z 71.15, 60.15, and 43.05 for MET; 235.10, 174.10, and 193.05 for SITA; 369.20, 169.00, and 304.15 for GLI; and for 153.10, 111.05, and 67.25 for PRM, which were used for quantification of these drugs in rat plasma.

As mentioned earlier, ethyl acetate has been used in this study as an extraction solvent in order to recover MET, SITA, and GLI from rat

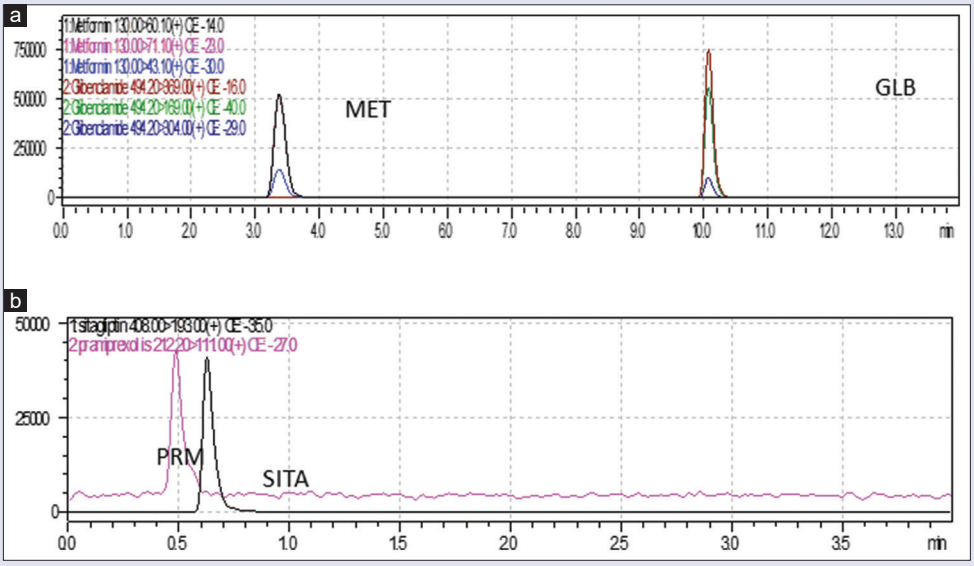


Figure 1: Analytical method for estimation of metformin, glibenclamide (a) and sitagliptin (b) in rat plasma. Metformin and glibenclamide used as internal standard for each other. Pramipexole used as internal standard for sitagliptin

plasma, which showed an extraction efficiency of 82.47%, 72.46%, and 79.23%, respectively. The calibration curves were found to show good linear correlation for the concentration ranging within 2.5–1000 ng/ml with r^2 = 0.9933 (MET), 0.9904 (SITA), and 0.9965 (GLI). The intra- and interday precision of the method ranged between 2.34%–4.65% and 3.52%–4.68% for all the three drugs with % relative standard deviation (%RSD) <10. The accuracy of the method was found within ±15%.

Effect of TCE on pharmacokinetics of metformin, sitagliptin, and glibenclamide in streptozotocin-induced diabetic rats

We studied the effect of TCE at dose 400 mg/kg on PK parameters of MET, SITA, and GLI after their individual and co-administration for 28 days in STZ-induced diabetic rats. Diabetic rats were used in order to assess the effect of diseased state on the PK of drugs. Moreover, comparing PK parameters of drug in the absence and presence of herb in the diseased animals is closer to real-life situation than studying them in normal animals. Few researchers have highlighted the importance of pathological conditions associated with disease and their effect on drug disposition and have proposed disease system approach for PK interaction studies.[38,39] In our study, we found no significant PK interaction between MET and TCE and SITA and TCE [Figure 2]. C_{max} and AUC of GLI were found increase 1.2 times in the presence of TCE; however, this interaction was considered clinically insignificant. As per the US FDA, strong, moderate, and weak inducers are drugs that decrease the AUC of the sensitive index substrates of a given metabolic pathway by ≥80%, ≥50% to <80%, and ≥20%. Similarly, strong, moderate, and weak inhibitors are drugs that increase the AUC of sensitive index substrates of the given metabolic pathway ≥5-fold, ≥2–5 fold, and ≥1.25 to <2-fold.

Pharmacodynamic interactions of TCE with metformin, sitagliptin, and glibenclamide in streptozotocin-induced diabetic rats

Fasting blood glucose

The fasting blood glucose (FBG) levels in the DC group were found to be significantly elevated as compared to NC ($P < 0.001$). Oral administration of TEC (400 mg/kg), MET (90 mg/kg), SITA (10 mg/kg), and GLI (1 mg/kg) significantly reduced the FBG levels to 252.9, 192.6, 221.1, and 227.8 mg/dl, respectively. However, the combination treatment provided more adequate glycemic control than either of the drugs or the herb alone suggesting synergy between them. The concomitant treatment with TC + MET, TC + SITA, and TC + GLI for 28 days reduced the FBG levels to 148.8, 147.4, and 188.5 mg/dl, respectively. Moreover, none of these combinations led to any incidence of hypoglycemia, which may occur as an adverse event in case of overdose of antidiabetic drug combination [Figure 3a].

Aspartate aminotransferase and alanine aminotransferase

Blood levels of AST and ALT are considered as indicators of liver damage. Patients of T1DM may suffer from chronic liver injury and nonalcoholic fatty liver, associated with the increased levels of AST and ALT.[40] Increased oxidative stress in chronic diabetes is considered to be one of the contributing factors toward hepatic damage. We found significantly elevated levels of AST and ALT in the DC group. However, TCE significantly reduced the elevated levels of these enzymes. In case of combination treatments, these levels reduced as compared to individual drug treatment [Figure 4a].

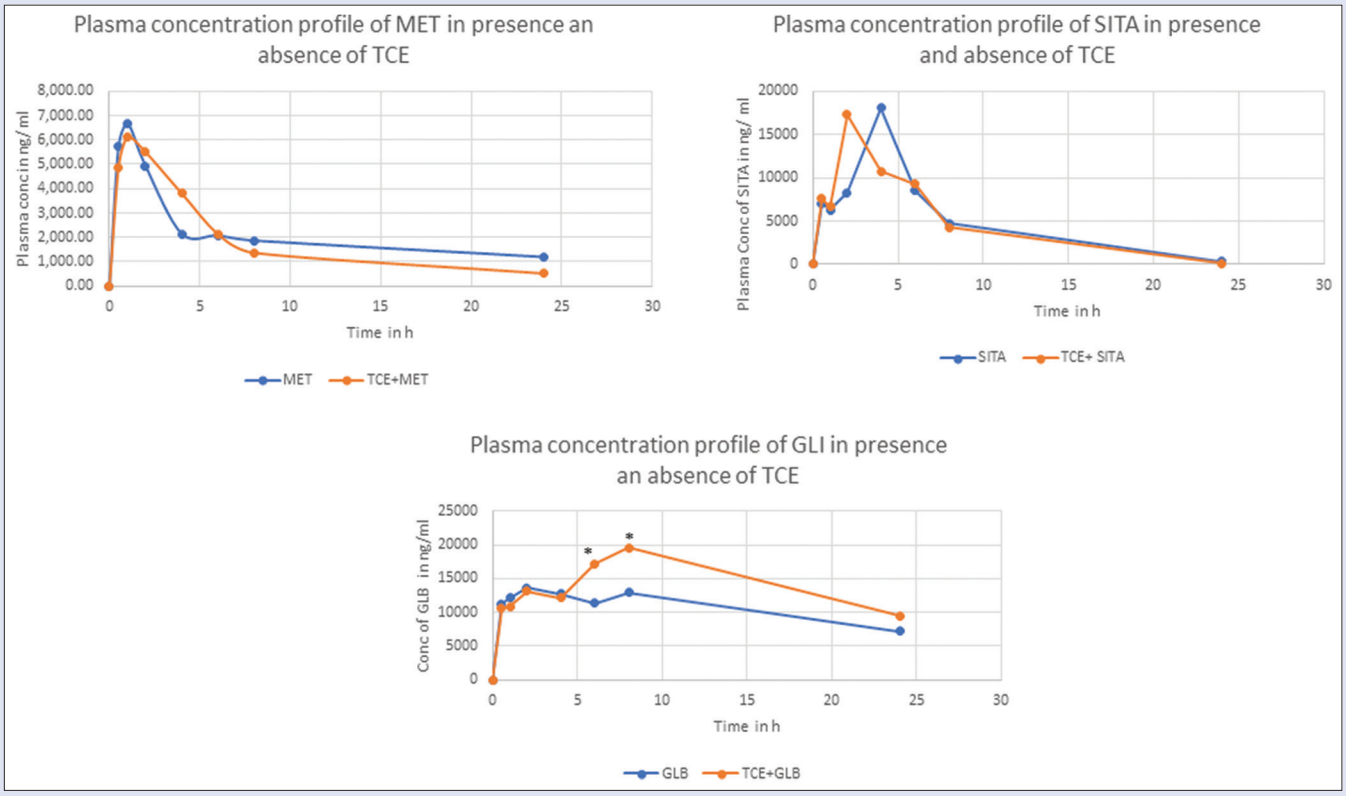


Figure 2: Plasma concentration profiles of MET (90 mg/kg), SITA (10 mg/kg), and GLI (1 mg/kg) alone and on concurrent treatment with TCE (400 mg/kg), *Significant with respect to drug alone treatment group at $P < 0.05$ ($n = 6$). TCE: *Tinospora cordifolia* extract; MET: Metformin; SITA: Sitagliptin; GLI: Glibenclamide

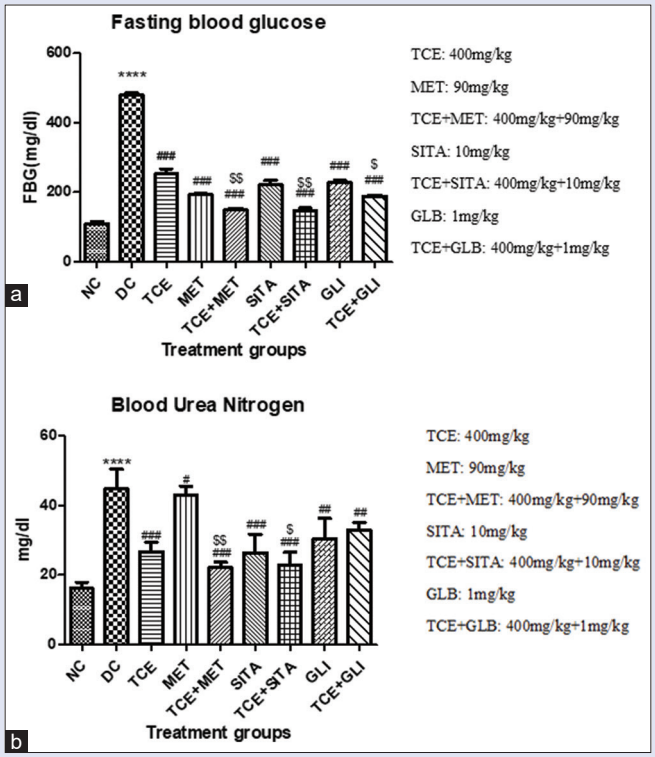


Figure 3: Effect of TCE, MET, TCE + MET, SITA, TCE + SITA, GLI, and TCE + GLI on (a) FBG and (b) blood urea nitrogen. Results are expressed as mean ± standard error of mean of six rats. *Significant with respect to normal control group, #Significant with respect to disease control group, $Significant with respect to respective drug alone treatment group at $P < 0.01$. TCE: *Tinospora cordifolia* extract; MET: Metformin; SITA: Sitagliptin; GLI: Glibenclamide

Total cholesterol and triglyceride

Dyslipidemia is one of the associated symptoms of diabetes mellitus and is known to increase cardiovascular risk.[41,42] Effective control of blood glucose level can reduce the chances of diabetes-associated microvascular complications such as diabetic nephropathy, neuropathy, and cardiomyopathy. In order to reduce the incidence of such symptoms, an effective lipid management needs to be adopted by patients with diabetes. This experimental analysis of the STZ-induced diabetic group of rats showed a significant increase in their TC level. Although MET and SITA reduced TC and TG significantly, reduction with TCE + MET, TCE + SITA, and TCE + GLI significantly decreased the TC and TG levels (with $P < 0.001$) to normal in the STZ-induced diabetic rats [Figure 4b].

Blood urea nitrogen

BUN is widely used as a marker for assessing kidney function. The BUN level is elevated significantly to 44.76 ± 5.26 IU/dl in diabetic control animals. Although there was a significant improvement in the levels of BUN with MET, SITA, and GLI, treatment with TCE, TCE + MET, TCE + SITA, and TCE + GLI for 28 days improved the elevated levels of BUN in diabetic rats [Figure 3b].

Food intake, water intake, and urine output

The three main symptoms of diabetes mellitus include polyuria, polydipsia, and polyphagia. The physical parameters of the STZ-induced diabetic rats showed a significant increase in the food and water intake as well as urine output (with $P < 0.001$). Treatment with MET and SITA reduced the food intake, water intake, and urine output in diabetic rats; however, GLI did not show a significant effect on the same. Treatment with TCE, TCE + MET, TCE + SITA, and TCE + GLI showed a significant reduction in increased food intake, water intake, and urine output in diabetic rats [Figure 5].

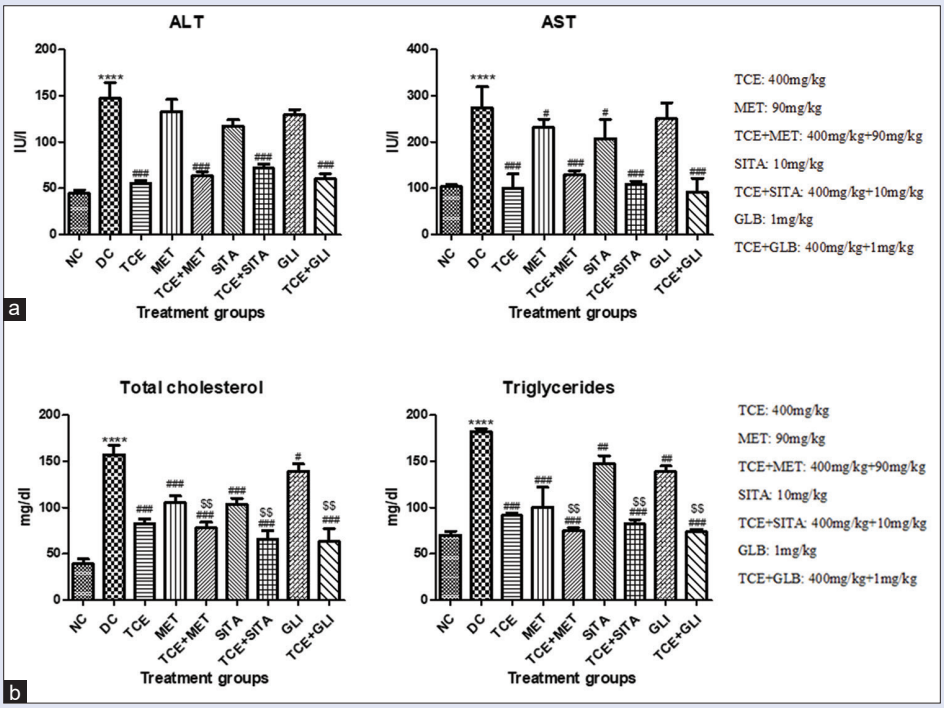


Figure 4: Effect of TCE, MET, TCE + MET, SITA, TCE + SITA, GLI, and TCE + GLI on (a) alanine transaminase and aspartate transaminase, (b) total cholesterol and triglyceride. Results are expressed as mean ± standard error of mean of six rats. *Significant with respect to normal control group, #Significant with respect to disease control group, $Significant with respect to respective drug alone treatment group at $P < 0.01$. TCE: *Tinospora cordifolia* extract; MET: Metformin; SITA: Sitagliptin; GLI: Glibenclamide

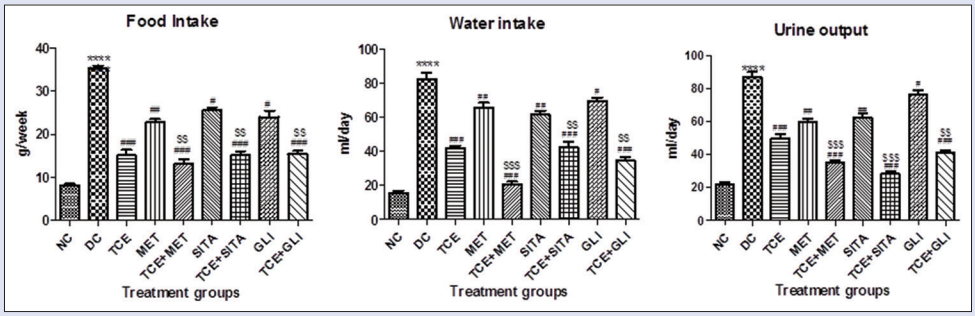


Figure 5: Effect of TCE, MET, TCE + MET, SITA, TCE + SITA, GLI, and TCE + GLI on food and water intake and urine output. Results are expressed as mean ± standard error of mean of six rats. *Significant with respect to normal control group, #Significant with respect to disease control group, $Significant with respect to respective drug alone treatment group at $P < 0.01$. TCE: *Tinospora cordifolia* extract; MET: Metformin; SITA: Sitagliptin; GLI: Glibenclamide

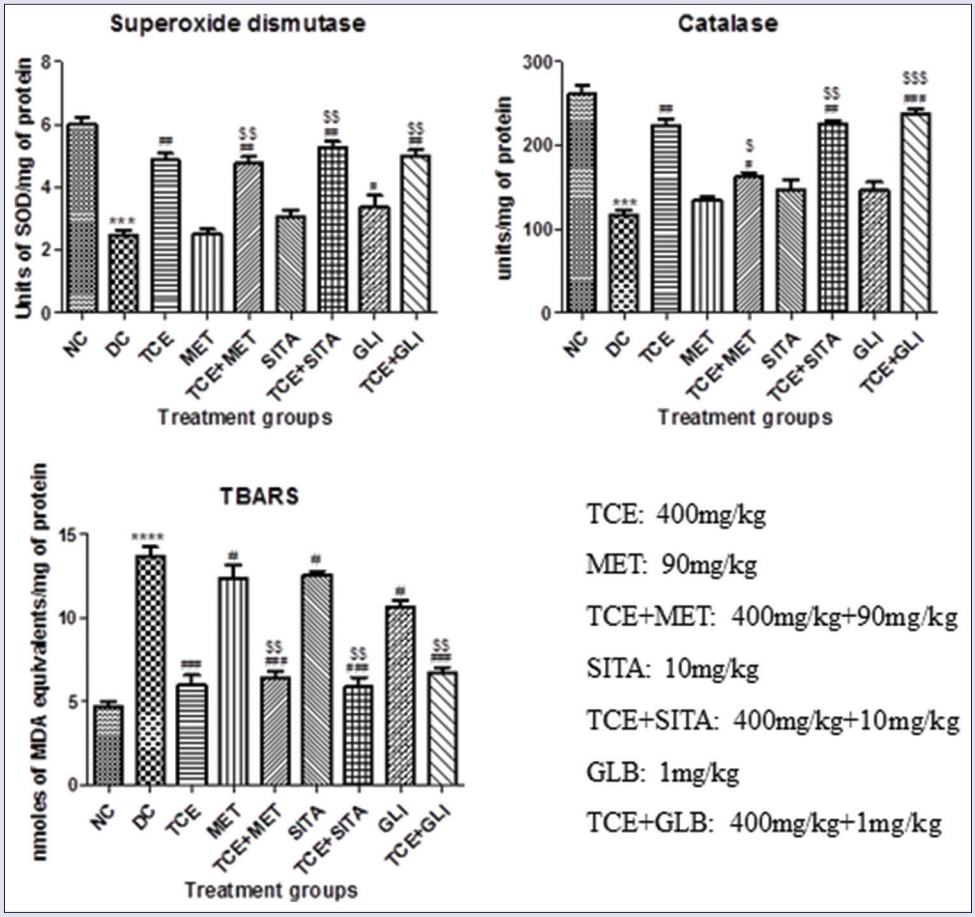


Figure 6: Effect of TCE, MET, TCE + MET, SITA, TCE + SITA, GLI, and TCE + GLI on SOD, catalase, and TBARS in pancreatic tissue. Results are expressed as mean ± standard error of mean of six rats. *Significant with respect to normal control group, #Significant with respect to disease control group, $Significant with respect to respective drug alone treatment group at $P < 0.01$. TCE: *Tinospora cordifolia* extract; MET: Metformin; SITA: Sitagliptin; GLI: Glibenclamide

Antioxidant activity in pancreatic tissue

Treatment with STZ significantly reduced the levels of antioxidants enzymes such as SOD, CAT, Reduced glutathione (GSH), and GPx up to 45.2%, 53.6%, 38.3%, 40.2%, and 35.9%, respectively, and elevated levels of TBARS in the pancreatic tissue by 2.5 times as compared with the NC groups. Treatment of animals with MET, SITA, and GLI had no significant effect on these antioxidant enzymes. However, treatment of animals with TCE and the combination of TCE + MET, TCE + SITA, and TCE + GLI significantly reduced the effect of STZ by bringing the levels down to physiological levels recorded in the normal group [Figures 6 and 7].

Histopathological examination of pancreatic tissue

The histopathological examination of rats belonging to the NC group showed normal histology (Grade 0). However, the pancreatic tissue of the diabetic control group animals reported severe injury leading and necrosis of the adipose tissues and macrophage infiltration with cytoplasmic vacuolation at endocrine pancreas (Grade ++++) with decreased islet cell count. Treatment with hypoglycemic drugs (MET, SITA, and GLI)

for 28 days reported a moderate level of tissue damage (Grade ++). Treatment with TCE and combination of TCE with MET and SITA and GLI for 28 days revealed that there was a better recovery of the pancreatic tissue in comparison with the individual drug treatment [Figure 8].

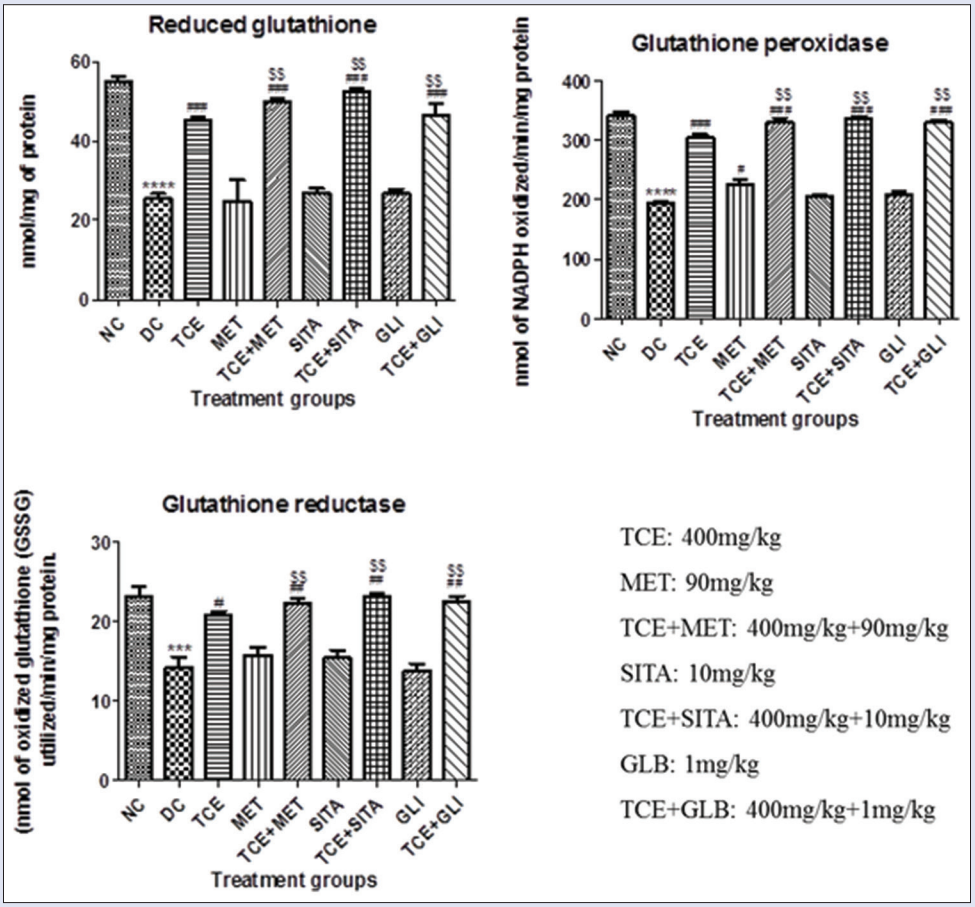


Figure 7: Effect of TCE, MET, TCE + MET, SITA, TCE + SITA, GLI, and TCE + GLI on Glutathione system of pancreatic tissue. Results are expressed as mean ± standard error of mean of six rats. *Significant with respect to normal control group, #Significant with respect to disease control group, $Significant with respect to respective drug alone treatment group at $P < 0.01$. TCE: *Tinospora cordifolia* extract; MET: Metformin; SITA: Sitagliptin; GLI: Glibenclamide

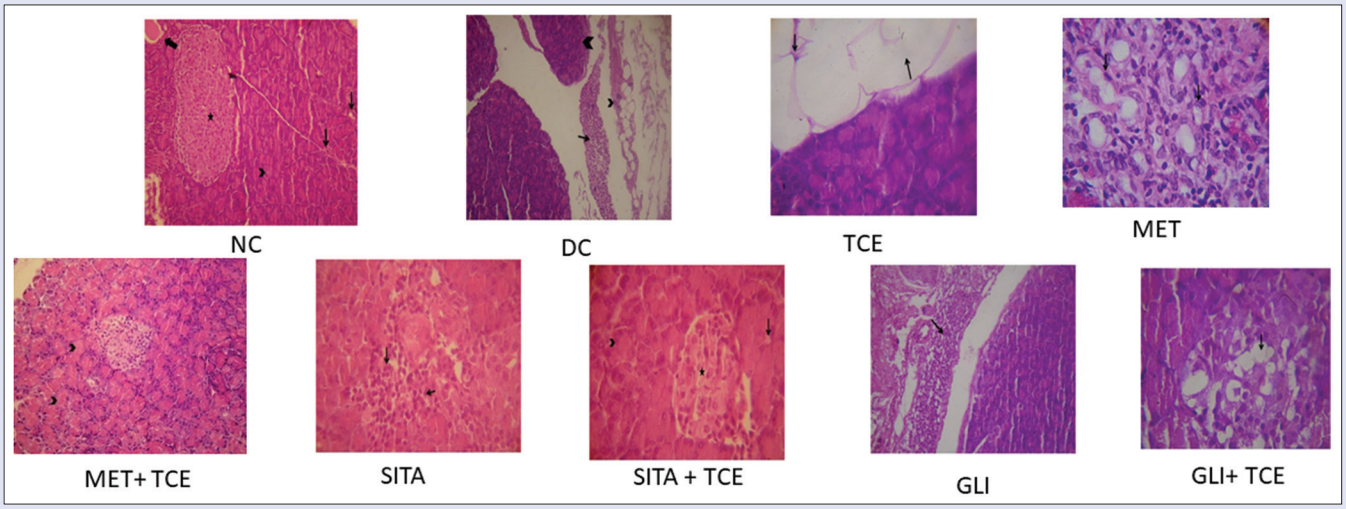


Figure 8: Histopathology of pancreatic tissue. NC: normal acinus (arrowhead), normal interlobular duct (large arrow), and normal islet of Langerhans (star); DC: Necrosis of adipose tissues (small arrow) and macrophage infiltration (arrow head) with cytoplasmic vacuolation at endocrine pancreas (arrow); TCE: normal acinus with mild lymphocytic infiltration (small arrow) and Focal minimal cytoplasmic vacuolation of endocrine pancreas; MET: atrophy of acini (small arrow); TCE + MET: Normal acinus with eosinophilic foci (arrow head); SITA: normal acinus with mild focal minimal lymphocytic infiltration (small arrow); TCE + SITA: normal acinus (arrow head) with mild lymphatic infiltration, normal intercalated duct (arrow), and normal islet of Langerhans (star) GLI: atrophy of acini (small arrow) with lymphocytic infiltration; TCE + GLI: normal acinus with mild focal minimal lymphocytic infiltration (small arrow)

DISCUSSION

For centuries, medicinal plants have been widely used for treating symptoms of chronic diseases such as diabetes, obesity, and various neurodegenerative disorders. According to WHO (2016), nearly 70% of the global population use herbal extracts as an alternative medicine source.[43] These herbal products are available over the counter, and patients may knowingly or unknowingly consume them along with the prescribed allopathic medicines. There are numerous reported cases of herb–drug interactions where the outcome could be increased or decreased therapeutic response or toxicity. The herb–drug combination may result in beneficial or harmful interaction.[44]

The animal model chosen in the present study is STZ-induced diabetic rats. STZ at 55 mg/kg produces T1D by DNA alkylation-induced generation of reactive oxygen species and enhanced formation of nitric oxide (NO) in the beta-cells of the pancreas. Although the primary treatment for T1DM includes insulin, MET, SITA as well as GLI are used as add-on therapies to reduce the requirement of insulin. Moreover, T1DM is known to be associated with an enhanced risk of comorbidities such as obesity, fatty liver, glycogenic hepatopathy, diabetic nephropathy, and micro- and macrovascular complications.[45]

In the present work, we studied the PD and PK parameters of three widely and commonly used oral hypoglycemic drugs belonging to different classes, i.e., MET, SITA, and GLI in the presence and absence of TCE (400 mg/kg), administered for a period of 28 days. The study of PK parameters of MET, SITA, and GLI indicated no significant alterations when given in combination with TCE. However, the combination of treatments exhibited significantly better therapeutic response in terms of glycemic control. Moreover, the associated parameters of the liver, kidney, and lipid profile showed a significant improvement in case of the concurrent treatment, and no cases of hypoglycemia were reported.

The significantly enhanced PD activity may be attributed to different mechanisms by which the drugs and the herb act. MET acts by reducing hepatic production and intestinal uptake of glucose, SITA acts by inhibiting DPP-4, and GLI acts by inhibiting ATP-dependent potassium efflux from pancreatic beta-cells. However, the effect of TCE may be due to the recovery of pancreatic beta-cells and their antioxidant status. This is in agreement with the previously reported antioxidant capacity of TCE.

CONCLUSION

The combination therapy, including MET + TCE, SITA + TCE, and GLI + TCE, negated the possibility of any form of adverse effect of the herb–drug combinational treatment. Hence, such experiments are safe to be conducted in clinical setups. However, the results of this study do not rule out the possibility of drug interactions of *T. cordifolia* in patients under treatment with both insulin and oral antihyperglycemic drugs which should be considered in future clinical studies on this topic. The benefit of these combinations lies in significant improvement in the control over glycemia and diabetic comorbidities in comparison to the single treatments with these drugs.

Acknowledgements

The authors would like to acknowledge the Central Council for Research in Unani Medicine, Ministry of AYUSH, Government of India, for funding this project (Project No.-3.28/2014-CCRUM/Tech).

Financial support and sponsorship

This research work was received from Central Council for Research in Unani Medicine, Ministry of AYUSH, Government of India, (Project No.-3.28/2014-CCRUM/Tech).

Conflicts of interest

There are no conflicts of interest.

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