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Eugenia jambolana extract reduces the systemic exposure of Sitagliptin and improves conditions associated with diabetes: A pharmacokinetic and a pharmacodynamic herb-drug interaction study

A. Vora [a, *], A. Varghese [a], Y. Kachwala [a], M. Bhaskar [a], A. Laddha [a], A. Jamal [b], P. Yadav [b]

[a] Shobhaben Pratapbhai Patel School of Pharmacy & Technology Management, SVKM's NMIMS, India
[b] Central Council for Research in Unani Medicine, Ministry of Ayush, Government of India, India

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ABSTRACT

Eugenia jambolana (EJ) is an Indian traditional herb widely used for the treatment of diabetes mellitus. This herb is globally marketed as single or multi herb formulations. Many diabetes patients consume EJ extract oral hypoglycemic drugs together. This calls for a need to assess risks versus benefit of this co-administration.

In present investigation, pharmacodynamic and pharmacokinetic interactions of aqueous extract of EJ seeds at the dose of 400 mg/kg are studied with 10 mg/kg of oral hypoglycaemic drug sitagliptin (SITA) by co-administrating them for 28 days in streptozotocin (STZ) induced diabetic rats. The pharmacokinetic parameters of SITA were determined using HPLC-ESI-MS/MS and it was found that the combination treatment reduces the systemic exposure of SITA by showing 38.70% reduction in concentration maximum (Cmax) and 22.40% reduction in area under curve (AUC). Despite low levels of SITA, the combination demonstrated a significant reduction in blood glucose level when compared with individual drug and individual extract administered groups during pharmacodynamic study. In addition, the liver function, the kidney function and the lipid parameters were found to be significantly improved and beneficial effects were found with respect to food intake and water intake and urine output in case of combination treatment groups when compared with individual treatment groups. Histopathological examination of pancreatic tissue suggests its significant recovery of having normal acinus with better cell protection in combination treatment. In conclusion, the combination treatment demonstrated reduced systemic exposure of SITA without compromising on its antihyperglycemic activity and improvement in conditions associated with diabetes.

1. Introduction

Jamuns (*Eugenia jambolana* Lam.) (EJ) are considered to be functional foods owing to their anti-diabetic[1] and anti-ulcer effects.[2] The aqueous extract of dried seeds of *Eugenia jambolana* (EJE) is globally marketed as monotherapy or constituent of poly-herbal formulations for the treatment of diabetes. Many patients consume EJE as home remedy along with standard treatment with oral hypoglycemics for the better management of this chronic metabolic disorder. However, the implications of their concomitant use are not well documented. The possible occurrence of herb-drug interaction leading to enhanced or diminished effect of one or both components needs to be investigated.

Several research groups studying the protective and therapeutic effect of EJE and have identified presence of various classes of bioactive constituents like anthocyanins, flavonoids[1], ellagitannins, gallotannins,[1] carotenoids[1] and considered them to be responsible for its wide array of activities like anti-hyperglycemic,[2] anti-hyperlipidemic,[3] antimicrobial, and antioxidant.[4] Recently, Yuanyuan, Li, et al., 2017 have isolated four different triterpenoids and have shown their relation with the antihyperglycemic activity of the fruit.[5] The putative anti-diabetic mechanism for EJE is ascribed to possible regeneration of islets cells, increase in peripheral

* Corresponding author.
E-mail address: amisha.vora@nmims.edu (A. Vora).
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utilization of glucose, increase insulin secretion, inhibition of glucose absorption from the intestine, modulation of the activity of intestinal glucose transporter and carbohydrate hydrolysing enzymes α-amylase and α-glucosidase,[6] correction of hepatic hexokinase I enzyme levels, inhibition of sucrase and maltase.[7] EJE is known to recover serum insulin levels and glycated haemoglobin levels in streptozotocin induced diabetic animals.[7] Due to potential of this herb in reducing diabetic condition, it may be knowingly or unknowingly used along with oral hypoglycemic drugs for management of diabetes. Hence, it is imperative to study the effect of co-administration of this herb on the pharmacokinetics and pharmacodynamics of commonly used antidiabetic drugs.

Gliptins are a class of oral anti-diabetic agents which act by competitively antagonizing the enzyme dipeptidyl peptidase-4 (DDP-4). The first agent of this class is Sitagliptin (SITA), approved by FDA in 2006. It is known to be transported by human organic anion transporter (hOAT3), organic anion transporting polypeptide (OATP4C1) and multidrug resistance (MDR) P-glycoprotein (P-gp).[8] Only 16% of the administered dose gets metabolised by CYP3A4 and CYP2C8 and the rest gets eliminated unchanged via kidney.[9] These transporter proteins and metabolising enzymes are known to be vulnerable to inhibition or induction by herbs leading to potential herb-drug interaction.

Various herb drug interaction studies have been carried out in past with respect to antidiabetic activity and Gupta et al. (2017) have published a complied review of the same.[10] However, to the best of our knowledge such studies have not been carried out for concurrent administration of EJE and SITA. Herb–drug interactions have been described as 'double-edged sword' presenting both risks (via adverse drug events or decreased therapeutic efficacy) and benefits (via enhanced therapeutic effect or reduced systemic exposure of drug which may result in lower incidence of side effects).

The present work was carried out to check the safety and efficacy of one of the probable concomitant treatments that a diabetic patient is likely to undertake during the course of his treatment. It evaluates the effect of sub-chronic (28 days) administration of EJE on the pharmacokinetic (PK) and pharmacodynamic (PD) activity of one of the widely used oral hypoglycemic, sitagliptin (SITA) in streptozotocin induced diabetic rats.

2. Materials and method

2.1. Drug, chemicals and solvents

SITA was a gift from by U.S Vitamins, Mumbai, India and STZ was purchased from Sigma Chemical Co, St Louis, MO, USA., Erba Diagnostic kits like Glucose oxidase peroxidase (GOD-POD), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total cholesterol (TC), Triglyceride (TG), Blood urea nitrogen (BUN) were purchased from Nobel Diagnostics, Mumbai, India. Ammonium acetate, ammonium formate, citric acid, tri-sodium citrate and EDTA were purchased from Qualigens fine chemicals, Mumbai, India. LC-MS grade solvents methanol, acetonitrile and formic acid were procured from Merck® India Ltd. Double distilled water was used wherever applicable and other chemicals and solvents used were of analytical grade.

2.2. Plant material

Fresh EJ seeds were ordered and procured from Borivali National Park, Mumbai, India. Taxonomical identification and authentication of specimen was done at Agharkar Research Institute, Pune, India and a voucher specimen no.15–237 was deposited.

2.3. Extraction

Dried seed of *Eugenia jambolana* were powdered and used for preparation of aqueous extract by using the double maceration technique. Powdered seed (500 g) were macerated with 5L of distilled water for seven days. After seven days, the extract was filtered and the filtrate was stored in refrigerator until next processing. The marc was further macerated with fresh distilled water for additional seven days. It was filtered and the filtrate obtained in this step was mixed with the previous filtrate. The combined filtrates were concentrated by spray drying at 75° C to yield the dry powder extract.

2.4. Standardization of aqueous extract of EJ

EJ extract was standardised for the content of ellagic acid using previously reported high performance liquid chromatography method Farrukh A and co-worker[11]

2.5. Animals

Male Sprague Dawley rats, weighing 180–220 g were maintained at animal facility of Shri Vile Parle Kelvani Mandal's, Narsee Monjee Institute of Management Study (SVKM'S NMIMS), Mumbai, India. The animals were housed in clean polypropylene cages under standard conditions of humidity (50 ± 5%), temperature (25 ± 2 °C) and light (12 h light/12 h dark cycle) and fed with a standard pellet diet and water *ad libitum*. All animals were handled with human care and all the procedures were performed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Animal Welfare Division, Government of India, New Delhi, and protocol was approved by Institutional Animal Ethical Committee Animal House (Registration No. 1830/PO/Re/S/15/CPCSEA).

2.6. Pharmacokinetic (PK) interaction of EJ with SITA in diabetic rats

2.6.1. Induction of diabetes

Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared STZ (55 mg/kg b.w) in 0.1 M citrate buffer (pH 4.5) at a volume of 1 mL/kg bw. After 72 h, blood was collected from each rat from retro orbital plexus, plasma was separated by centrifugation at 4000g. Glucose level was determined using GOD-POD Erba diagnostic kits. Rats with fasting blood glucose level above 250 mg/dL were considered to be diabetic and selected for the further study.

2.6.2. Animal study

The diabetic rats were randomly divided into five groups of six animals each. *Group I* consisted of non-diabetic rats, (Normal Control- NC) administered with distilled water (1 ml/kg, *p.o*) daily for 28 days. *Group II* consisted of 55 mg/kg STZ induced diabetic rats (Diabetic control- DC). *Group III* diabetic animals were treated with SITA alone for 28 days at dose of 10 mg/kg *p.o* and *Group IV* diabetic animal were administered with EJE for 28 days at dose 400 mg/kg, *p.o. Group V* diabetic animals received EJE (400 mg/kg) and SITA (10 mg/kg) for 28 days. The solutions of SITA and EJE were freshly prepared in distilled water and administered via oral route using an oral gavage needle. In Group V animals, SITA was administered first, followed by EJE. LD_{50} of EJE is reported to be > 5000 g/kg.[12] The doses of SITA and EJE were calculated by applying the conversion factor to their respective human effective dose on the basis of body surface area of rats.[13,14]

2.6.3. Pharmacokinetic study

On the 28th day, after the treatment, blood was collected in heparinised tubes from retro-orbital plexus (0.4–0.5 mL) from group III and V at predetermined time interval 0, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h. The animals were anaesthetized using isoflurane during blood withdrawal. The time intervals were selected with commonly used strategies for pharmacokinetic studies i.e. the total blood withdrawal should not be over 20% of the total blood volume. Blood samples were centrifuged at 4000g for 10 min at 4 °C to obtain the plasma which was stored at −80 °C until analysis using HPLC-ESI-MS/MS method. The pharmacokinetic parameters such as concentration maximum (C_{max}), area under the plasma concentration-time curve (AUC), terminal elimination half life ($t_{1/2}$) were estimated using pharmacokinetic program WinNonlin® version 3.0 (Pharmasight corporation, Mountain view, CA). The maximum plasma concentration (Cmax) and the time to reach C_{max} (T_{max}) were obtained directly from the plasma concentration-time curve. All values were expressed as mean ± SEM. T_{max} was mentioned as a median.

2.6.4. Liquid chromatography and mass spectrometric condition

HPLC-ESI-MS/MS method was developed for quantification of SITA in rat plasma using pramipexole (PRM) as an internal standard (IS). The liquid chromatography system (Shimadzu, 8040) consisted of a binary LC-20AD prominence pump, an autosampler (SIL–20 AC_{HT}), an online solvent degasser (DGU-$20A_{5R}$) and a temperature-controlled compartment for column (CTO-20AC). The chromatographic separation was achieved on Kromasil C_{18} (4.6 × 50 mm, 3.5 μm particle size; Kromasil, SE-44580, Bohus, Sweden) analytical column maintained at 40 °C temperature. The elution was carried out using 0.1% formic acid and methanol (40:60) as mobile phase under isocratic mode and the flow rate was maintained at 1 mL/min. The total run time was set to 4 min. The detection of analyte (SITA) and IS (PRM) was carried out on a triple quadrupole mass spectrometer, equipped with electrospray ion source in the positive ion mode. The lab solutions software version 5.75 was used to control all parameters of LC and MS. The quantification was performed using multiple reaction monitoring (MRM) mode, based on parent → product ion transitions for SITA and PRM. The source dependent parameters were optimized at gas 1 (nebuliser gas): 3 L/m; gas 2 (drying gas): 15 L/m; ion spray voltage (ISV): 4500 V; heat block temperature at 400 °C and DL temperature: 250 °C. The collision energy (CE) was set at −35 for sitagliptin and −27 and −38 for pramipexole. Nitrogen was used as collision activated dissociation (CAD) gas and was set at 230 kPa. Quadrupole 1 and quadrupole 3 were maintained at a unit resolution and dwell time was set at 100 ms.

2.6.5. Preparation of stock, working dilution and spiking solution and determination of SITA linearity in rat blank plasma

The standard stock solutions of SITA and the PRM were prepared by dissolving their accurately weighted amounts in methanol to get a final concentration of 1000 μg/mL. This was further diluted to prepare the working solution of SITA in the concentration range of 0.025–25 μg/mL and for PRM at the concentration of 10 μg/ml using methanol as diluent. The solutions were stored at 2–8 °C and were brought to room temperature before use. The calibration standards were prepared by spiking 90 μL of blank rat plasma with 10 μL respective working solutions to produce the final concentrations of 25, 50, 100, 250, 500, 1000, 2500, 5000, 10000, 25000 ng/mL for SITA. The quality controls standards were prepared at 25 ng/mL (low limit of quality control, LLOQ, 100 ng/mL (low quality control, LQC), 1000 ng/mL (middle quality control, MQC) and 10000 ng/mL (high quality control, HQC). 10 μL PRM was added as an internal standard final concentration of 1000 ng/mL to each of the samples. 1000 μL of ethyl acetate was added as an extraction solvent to each of the samples. After vortexing for 10 min and centrifuging the samples at 4000g for 10 min, 900 μL of the supernatant was withdrawn, evaporated to dryness at 40 °C under nitrogen gas and reconstituted with 100 μL of mobile phase. 8 μL of the samples were injected into the HPLC-ESI-MS/MS.

2.7. Pharmacodynamic (PD) interactions in streptozotocin induced diabetic rats

For determination of pharmacodynamic parameters, blood was withdrawn from animals of each group after the last treatment dose on the 28th day and various biochemical parameters were analyzed.

2.7.1. Estimation of blood glucose parameters, biochemical parameters, food intake, water intake and urine output of animals

FBG and biochemical parameters like Alanine transaminase (ALT), Aspartate transaminase (AST), total cholesterol (TC), triglyceride (TG), and blood urea nitrogen (BUN) were estimated using ERBA diagnostic kits as per manufacturer's protocol. Food intake, water intake and urine output were determined using metabolic cages.

2.7.2. Histopathology of rat pancreas

At the end of 28 days, rats from each treatment group were sacrificed, the pancreas were isolated and rinsed with ice cold saline. Then the tissues were fixed with 10% formaldehyde. The tissue histopathology was carried out at Chaitanya pathology laboratory, Pune, India. The sections were stained with hematoxylin and eosin (H & E) and were examined at least at three different sites under a light microscope for histo-architectural changes. The pathological grading was done on the basis of the extent of necrosis of islet cells.

2.8. Statistical analysis

The result of pharmacodynamic study was expressed as mean ± standard error of mean (SEM) of six animals from each group. Graph Pad prism Version 6.00 of Graph Pad Software Inc., San Diego, USA, software was used for statistical analysis and the results were analyzed using one-way ANOVA followed by the Tukey–Kramer post test; $p < 0.001$ was considered as level of significance for pharmacodynamic parameter. Pharmacokinetic parameters were analyzed using two-way ANOVA followed by the Tukey–Kramer post test; C_{max} and AUC of treatment group were compared with diabetic control animals and diabetic animals were compared with normal control animals. $p < 0.05$ was considered as level of significance.

3. Results

3.1. Standardization of EJ aqueous extract

The content of ellagic acid in the aqueous extract of EJ seeds by HPLC was found to be 0.37%w/w. Ellagic acid is a known polyphenolic antioxidant and is reported to reduce blood sugar in diabetic rats.

3.2. PK interactions of EJ extracts with SITA

3.2.1. HPLC-ESI MS/MS method for SITA

HPLC-ESI MS/MS method was developed and validated for determination of SITA in rat plasma samples. The retention time for SITA and PRM were found to be 0.642 and 0.483 min respectively (Fig. 1a) and their masses was confirmed at *m*/*z* 408.04 and 212.20

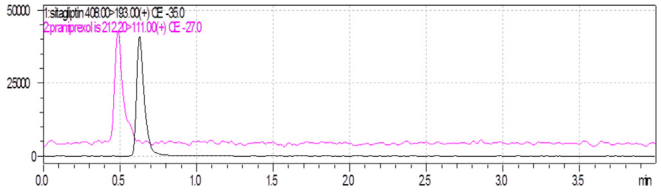

Fig. 1. Mass chromatograms of SITA and PRM (Peak for SITA at 0.642min, PRM at 0.482 min).

(Fig. 2a and b). Further collision induced fragmentation of SITA led to product ions of *m/z* 193.0, 174.0, 154.0 and that of PRM led to product ions of *m/z* 111.0, 67.10 (Fig. 3a and b). These were used to set MRM transitions at *m/z* 408.04 → 193.0, 174.0, 154.0 for SITA and *m/z* 212.20 → 111.0, 67.10 for PRM for the purpose of their quantification in rat plasma.

3.2.2. *Extraction of SITA from rat plasma and sample preparation*

Ethyl acetate was used as extraction solvent to recover SITA and PRM from plasma. The extraction efficiency with ethyl acetate was found to be 72.46%. The method was validated for linearity, accuracy and precision. The calibration curves showed good linear correlation in the concentration range of 25–25000 ng/mL with $r^2 = 0.9933$. The lower limit of quantification was found to be 25 ng/mL. The intra and inter-day precision of the method ranged between 2.34 - 4.65% and 3.52–4.68% with % RSD <10. The accuracy of the method was found within ±15%. This method was used for quantitative analysis of SITA in rat plasma samples obtained from pharmacokinetic interaction studies.

3.2.3. *Effect of EJ extracts on SITA pharmacokinetics in STZ induced diabetic rats*

The effects of EJ extracts at a dose of 400 mg/kg on pharmacokinetics (PK) of SITA (10 mg/kg) were studied in STZ induced diabetic rats. Though PK interaction studies can be carried out in normal rats, we carried it out in diabetic rats to relate it with the real-life condition. Various recent researches have proposed the disease system approach as various diseases and pathological conditions associated with disease can modify the drug disposition.[15] The present study shows the comparative pharmacokinetic parameters of SITA alone and when given concurrently with EJ extract. The C_{max} was significantly reduced by 38.70% and $AUC_{0\text{-}24}$ by 22.40% ($p < 0.05$) in the group of rats with concurrent administration of SITA and EJ in comparison with SITA alone (Fig. 4, Table 1). In other words, the systemic exposure of SITA was significantly reduced when concurrently administered with EJE. These results may be due to overexpression of efflux transporters

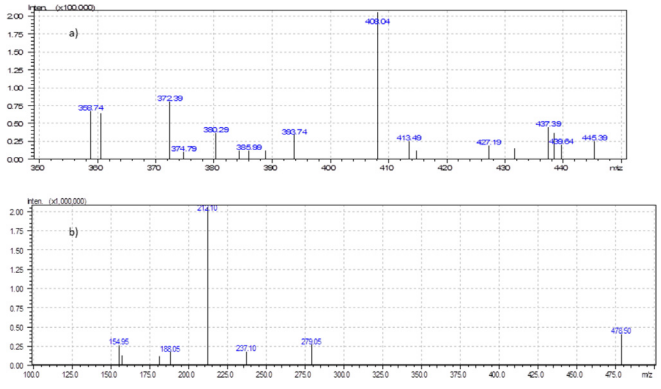

Fig. 2. a) Mass spectra of SITA at *m/z* 408.04, b) PRM at *m/z* 212.10.

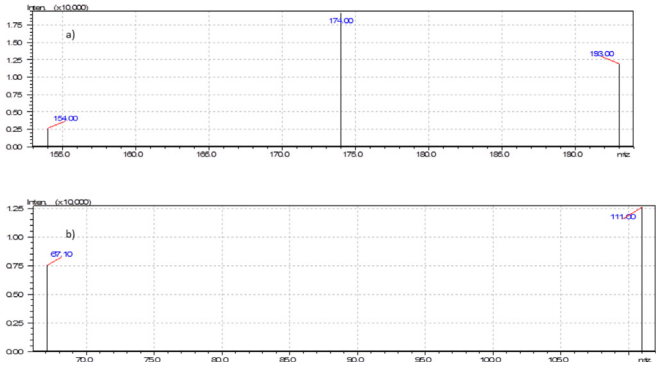

Fig. 3. a) Productions of SITA at *m/z* 198, 174, 154 b) PRM at *m/z* 111, 67.10.

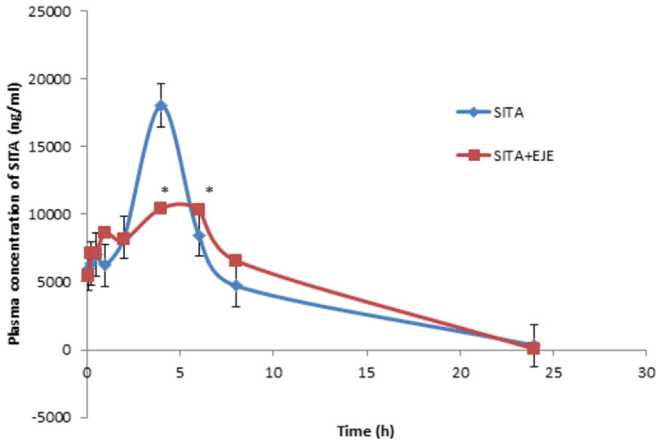

Fig. 4. Plasma concentration time profile of SITA in rats treated with SITA (10 mg/kg and SITA(10 mg/kg)+ EJE (400 mg/kg). Values expressed as mean ± SEM of six rats. *significantly different from SITA treated group at $p < 0.05$.

like intestinal P-gp, or induction of CYP metabolising enzymes known to be responsible for SITA metabolism, on prolonged treatment of 28 days with EJE. P-gp is a phosphorylated glycoprotein encoded by MDR1 gene responsible for systemic disposition of various structurally and pharmacologically unrelated xenobiotics. The role of P-gp is to protect the cell from various xenobiotics by the mechanism of causing its efflux. Overexpression of intestinal P-gp can lead to reduced plasma concentration of the drug.[16] Many herbs on prolonged treatment are known to overexpress these enzymes and modulate the systemic exposure of various drugs and chemicals proteins.[17–20] Further studies can be carried out to study the mechanism.

3.3. *Pharmacodynamic interactions of EJ and SITA in STZ induced diabetic rats*

3.3.1. *Effect of SITA, EJ and EJ + SITA on blood glucose levels*

The fasting blood glucose levels were significantly increased ($p < 0.001$) in diabetic rats as compared to normal control rats. Oral administration of SITA (10 mg/kg), aqueous extract of EJ seeds (400 mg/kg) and their combinations at respective doses for 28 days decreased the FBG significantly ($p < 0.001$) to 333, 321.86 and 298.74 mg/dL respectively as compared to diabetic control having fasting blood glucose 577.2 mg/dL (Fig. 5). It can be seen that despite low levels of SITA during pharmacokinetic interaction study, reduction in FBG levels was more significant with combination treatment as compared to SITA alone or EJE alone.

Table 1
Main Pharmacokinetic parameters of SITA in diabetic rats (n = 6) treated with SITA (100 mg/kg) and SITA + EJE (400 mg/kg).

Pharmacokinetic parameters	Animals treated with SITA alone	Animals treated with EJE + SITA
Cmax (ng/mL)	18916.15 ± 3837.346	11595.02 ± 1396.5*
Tmax (h)	2.85 ± 0.757	3.8 ± 1.02*
AUC (0–24 h)	113214.5 ± 8220.862	87851.076 ± 21620.072*
Elimination rate constant (ke)	0.184 ± 0.03	0.276 ± 0.05*
Elimination half-life ($t_{1/2eli}$) h	3.26 ± 0.54	2.14 ± 0.48*

Data expressed as mean + SD.
**significant with respect to SITA alone treated group.

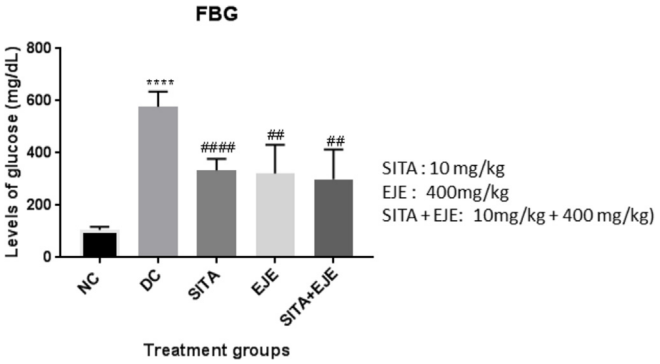

Fig. 5. Effect of SITA, EJE and SITA + EJE on Fasting blood glucose (FBG). Results expressed as mean ± SEM of six rats. *significantly different from normal group, # significantly different from diabetic control group at $p < 0.001$.

3.3.2. Effect of SITA, EJE and EJ + SITA on AST and ALT

Plasma levels of the enzymes ALT and AST are used biomarkers for liver damage. Diabetes mellitus is associated with elevated levels of AST and ALT. The underlying mechanism of diabetes that contributes to liver damage is the combination of increased oxidative stress and an aberrant inflammatory response.[21] Significant ($p < 0.001$) increase in the levels of ALT and AST were observed in plasma of diabetic rats indicating hepatic dysfunction. EJ extract has been shown to possess potent antioxidant property which can help in improvement of AST and ALT. SITA did not significantly help improvement of these parameters; however EJE and SITA + EJE treated groups showed significant reduction in elevated levels of these enzymes associated with diabetes.[19] (Fig. 6, Table 2).

3.3.3. Effect of SITA, EJE and EJE + SITA on total cholesterol (TC) and triglycerides (TG)

Diabetes is known to be associated with dyslipidemia and patients suffering from diabetic dyslipidemia are considered to be at 2–4 times higher risk of cardiovascular complications.

Diabetic dyslipidemia is characterised by elevated TC and TG. Reaching adequate blood glucose control is important in decreasing microvascular complications associated with diabetes; however, good lipid management is vital for reducing the incidence of cardiovascular events in patients with diabetes. The present study showed significantly increased level of total cholesterol in plasma of STZ induced diabetic group. Though SITA reduced TC and TG significantly, reduction with EJE and SITA + EJE was more significant ($p < 0.001$) bringing them down almost to normal in STZ induced diabetic rats (Fig. 7, Table 2).

3.3.4. Effect of SITA on blood urea nitrogen (BUN)

BUN is a reliable marker for kidney function. The levels of BUN were significantly elevated to 38.76 ± 2.3 IU/dL) in DC animals. Treatment with EJE and EJE + SITA significantly ($p < 0.001$) reduced the elevated levels of BUN (Fig. 8, Table 2).

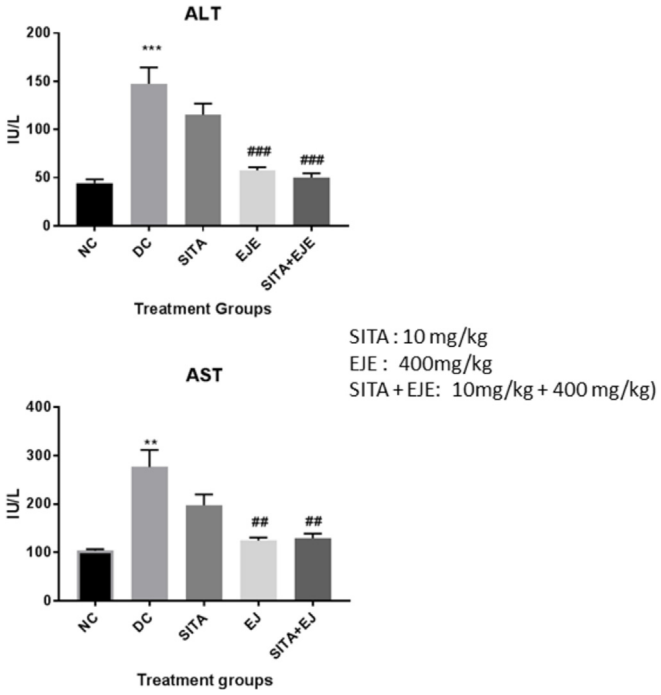

Fig. 6. Effect of SITA, EJE and SITA + EJE on Alanine transaminase (ALT) and Aspartate transaminase. (AST). Values expressed as mean ± SEM of six rats. *significantly different from normal group, # significantly different from diabetic control group at $p < 0.001$.

3.3.5. Effect of SITA, EJ and EJ + SITA on food intake, water intake and urine output

Polyuria, polydipsia, polyphagia are the three major symptoms of diabetes. STZ induced diabetic rats showed significant ($p < 0.001$) increased levels of food intake, water intake and urine output. Treatment with SITA reduced the elevated levels, however the effect was more significant ($p < 0.001$) in case of EJE as well as the combination treatment (Fig. 9, Table 2).

3.3.6. Effect of SITA, EJ and EJ + SITA on histopathology of pancreas

The histopathological study of normal control group reveals normal histology of the pancreatic tissue (Grade 0). Pancreatic tissue of DC group animals elicited severe injury showing necrosis of adipose tissues, macrophage infiltration with cytoplasmic vacuolation at endocrine pancreas (Grade ++++) with decrease in the number of islet cells. Treatment with SITA alone (10 mg/kg) for 28 days reduced the damage to a moderate level (Grade ++), whereas treatment with EJ alone for 28 days elicited recovery of pancreatic tissue as compared to SITA alone (Grade +++). Interestingly treatment with concomitant administration of SITA and EJ for 28 days elicited the significant recovery of pancreatic tissue (Grade +) having normal acinus with better cell protection than other treatment group (Fig. 10).

Table 2
Levels of ALT, AST, TC, TG, BUN, water intake, food intake and urine output in rats treated with SITA (100 mg/kg), EJE (400 mg/kg) and SITA + EJE (100 + 400 mg/kg).

Parameters	NC	DC	SITA	EJE	SITA + EJE
ALT	44.436 ± 3.42	147.34 ± 15.63***	115.33 ± 10.50	57.56 ± 3.12###	50.25 ± 4.03###
AST	103.65 ± 3.20	286.63 ± 36.91**	187.2 ± 22.80	140.3 ± 6.2##	19.02 ± 7.76##
TC	39.46 ± 4.54	157.2 ± 9.19***	121.83 ± 11.41##	63.16 ± 3.508###	61.20 ± 4.90###
TG	90.26 ± 18.42	182 ± 2.96***	147.66 ± 8.01##	117.66 ± 8.94###	88.13 ± 4.80###
BUN	16.14 ± 1.6	38.76 ± 6.94***	26.43 ± 4.89	16.42 ± 0.90#	15.57 ± 0.90#
Food intake	8.8 ± 1.13	36.86 ± 1.57***	25.6 ± 1.17#	15.8 ± 2.44###	11.8 ± 1.55###
Water intake	17.22 ± 0.83	92.12 ± 3.56****	67.23 ± 8.05##	42.19 ± 9.52###	31.74 ± 3.05###
Urine output	19.6 ± 3.18	95.18 ± 13.69***	46 ± 2.08#	38 ± 2.04##	27 ± 1.87##

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

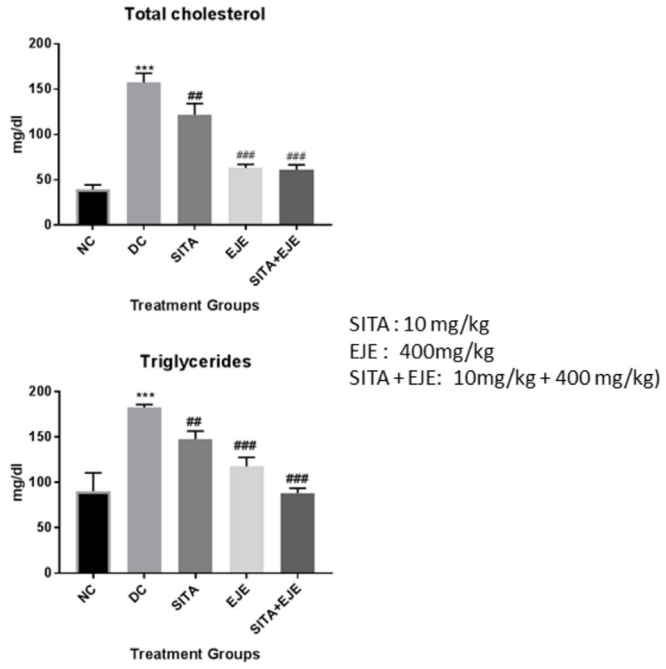

Fig. 7. Effect of SITA, EJE and SITA + EJE on total cholesterol and triglyceride. Values expressed as mean ± SEM of six rats. *significantly different from normal group, # significantly different from diabetic control group at $p < 0.001$.

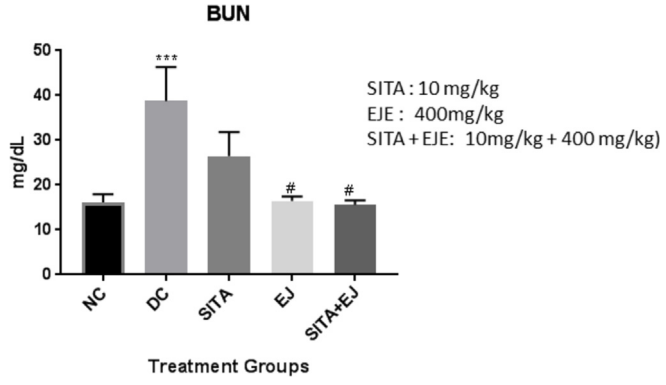

Fig. 8. Effect of SITA, EJE and SITA + EJE on Blood urea nitrogen (BUN). Values expressed as mean ± SEM of six rats. *significantly different from normal group, # significantly different from diabetic control group at $p < 0.001$.

44. Discussion

Diabetes is a complex metabolic disorder associated with

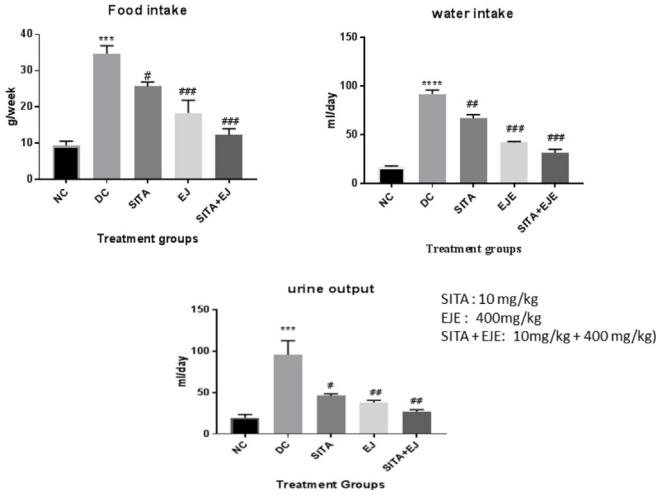

Fig. 9. Effect of SITA, EJE, SITA + EJE on food intake, water intake and urine output, Values expressed as mean ± SEM of six rats. *significantly different from normal group, # significantly different from diabetic control group at $p < 0.001$.

various comorbidities like neuropathy, nephropathy, retinopathy, liver damage and cardiovascular complications.[22] SITA is one of the commonly prescribed antidiabetic drug to be taken daily at dose of 100 mg/kg via oral route. Numerous clinical studies have provided evidence for use of DPP-IV inhibitors as therapeutic strategy for Type 1 diabetes mellitus[23] Few studies in animals have shown beneficial effects of *Eugenia jambolana* in Streptozotocin induced diabetes[7,24] There are various formulations of EJE in market and patients may take SITA and EJE simultaneously to control their diabetic condition. In such situation, it is important to study the safety and efficacy of this co-administration. Various herbs and fruit juices have been shown to modulate absorption, metabolism and elimination of drugs through various mechanisms involving efflux transporters, uptake transporters and metabolising enzymes.[25,26] In the present study, aqueous extract of EJ seeds was prepared, standardised it to the content of ellagic acid and administered at dose 400 mg/kg concomitantly with SITA 10 mg/kg for 28 days and evaluated the pharmacokinetic parameters of SITA using HPLC-ESI-MS/MS and compared them with that of SITA alone. Moreover, the pharmacodynamic activity of the combination as well as SITA alone and EJE alone are studied in terms of fasting blood glucose levels of alanine transaminase, aspartate transaminase, total cholesterol, triglyceride, and blood urea nitrogen, food intake, water intake and urine output.

Study of pharmacokinetic parameters indicated significantly reduced systemic exposure of SITA when given in combination with EJE as compared to SITA alone. Despite this, the therapeutic response in terms of FBG levels was not significantly affected. This

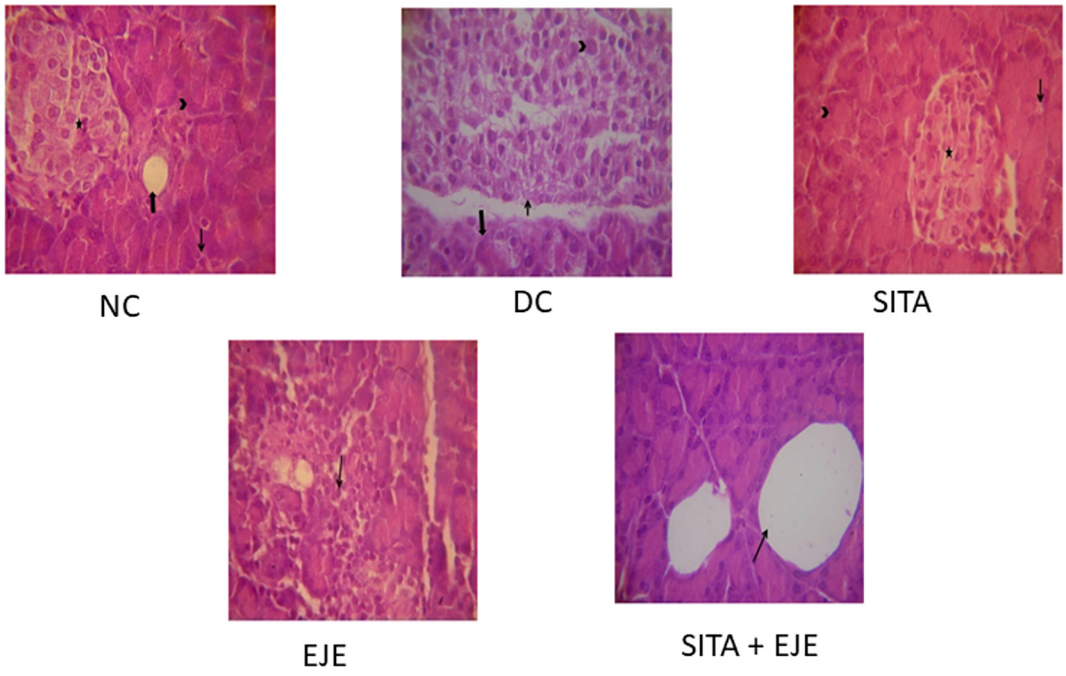

Fig. 10. Histopathology of pancreatic tissue.
NC:Showing normal acinus (arrowhead), normal interlobular duct (large arrow), and normal Islet of Langerhans (star).
DC: Showing necrosis of adipose tissues (small arrow), macrophage infiltration (arrow head) with cytoplasmic vacuolation at endocrine pancreas (arrow).
SITA: Focal minimal lymphocytic infiltration, Focal mild necrosis peri parenchymal adipose tissue (arrow head).
EJE: Focal minimal lymphocytic infiltration (small arrow).
SITA + EJE: Focal minimal lymphocytic infiltration (small arrow), normal acinus.

effect can be ascribed to two different mechanisms through which SITA and EJE act. The reduced systemic exposure of SITA can be beneficial in reducing its side effects without lowering its therapeutic efficacy when given concomitantly with EJE. Moreover, liver, kidney damage biomarkers, lipid profile, food and water intake, urine output and pancreatic cells damage showed prominent improvement in case of combination treatment when compared with SITA alone. Additionally, there were no incidences of hypoglycaemia involved in the combination treatment.

55. Conclusion

The combination SITA + EJ did not show any adverse herb drug interaction and is safe to be explored in a clinical set up. The benefit of the SITA + EJE combination lies in significant improvement in comorbidities associated with diabetes mellitus when compared with SITA alone treatment.

Declaration of interests

None.

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