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Food-drug interaction and pharmacokinetic study between fruit extract of *Capsicum frutescens L.* and glimepiride in diabetic rats

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Abstract

Background: The present study was designed to explore the food-drug and pharmacokinetic interaction between *C.frutescens*, a culinary herb on hypoglycemic activity of glimepiride, a sulfonyl urea derivative used in the treatment of type-2 diabetes) in diabetic rats in combination of each as single doses for one day. Further it is also aimed to study the effect of AQEFCF on antidiabetic effect of glimepiride by repeated dose treatment of AQEFCF for 8 days followed by single dose of Glimepiride in diabetic rats and also with repeated dose treatment of both drugs for 8 days in diabetic rats.

Methods: Acute toxicity study was conducted as per OECD guidelines 425, as per this study maximum 2000 mg/kg dose was given to albino mice as observed for mortality of the aqueous extract of *C.frutescens*. Later in order to know the dose dependent action three doses were selected (1/5th,1/10th and 1/20th) for antidiabetic study. Before treatment with either AQEFCF or Glimepiride fasting '0' blood samples were collected and serum glucose levels were analysed by GOD-POD method using semi-auto analyser. AQEFCF (100, 200 and 400 mg/kg p.o) glimepiride 1/2TD, TD and 2TD (0.036, 0.072 and 0.144 mg/200 g p.o) were administered orally alone as well as in combination i.e. AQEFCF as single dose followed by a single dose of glimepiride 30 min later in Phase I. In II Phase repeated doses of AQEFCF for 8 days followed by a single dose of glimepiride (30 min later) on 8th day. In Phase III both the drugs are administered as single doses for 8 days in the same group of diabetic rats. After the treatment serum glucose levels were determined in all the groups of rats at prefixed time intervals i.e.; 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24 h.

Results: Both AQEFCF and glimepiride when administered as single doses produced a dose dependent antidiabetic activity in diabetic rats. The combination of AQEFCF and glimepiride at the different dose levels has shown an better antidiabetic effect. AQEFCF augmented the effect of glimepiride in streptozotocin induced diabetic rats.

Conclusion: It has been concluded that no significant food drug interaction occurred between AQEFCF of *C. frutescence* and glimepiride either single dose or combination of repeated doses. Empirical evidences clears that there is also no pharmacokinetic interaction also observed.

Keywords: Food-drug interaction, *C.frutescens* fruit, Glimepiride, Streptozotocin, Diabetic rats, Serum glucose

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Background

Ayurvedic herbal preparations often consist of complex mixtures of plant materials practiced in many countries of the Indian subcontinent [1]. Herbal products are being used as a home remedies worldwide in a variety of healthcare settings and are often promoted to the public as being "natural" and completely "safe" alternatives to conventional medicines [2]. Diatery suppliments which contains single or mixture of herbs which are also known as botanicals, can be used for their flavor, scent and therapeutic properties [3]. A food/drug interaction occurs when a food, or one of its components interferes with the way a drug is acts in the body [4]. Food-drug interactions are more challenging (since food consumption is not documented on patient profiles), but often pose equally substantial risk of negative outcome. Interactions between food and drugs can have profound influence on the success of drug treatment and the side effect profiles of many drugs. Foods can interfere in a number of ways with drug action at different stages. The most common effect is of foods interference with drug at absorption level making the drug less effective [5]. In drug absorption process food place a vital role. In some cases the altering absorption mechanism remains unclear, In certain medications indigested food or liquid occasionally causes alteration in absorption [6]. The fruit extract of *Capsicum frutescens* L has already reported the presence of anti-diabetic activity [7]. Similarly Glimiperide is also a well known antidiabetic drug and which is already reported [8]. Based on the above fact, it came to our mind that, if any diabetic patient who is regularly consuming Glimiperide, what should be the effect of *Capsicum frutescens L* to the diabetic patient. So the present study was designed to observe the effect of *Capsicum frutescens* L with Glimepiride. Since no study was available on food drug interaction of capsicum with oral hypoglycemic drugs (Fig. 1), the present study was planned to ivestigate the food-drug interactions and pharmacokinetic study between a widely used dietary food preparations like *Capsicum frutescens* L, and a synthetic oral antidiabetic drug glimepiride, since both posses hypoglycemic activity.

Materials and methods

Plant material

The fruits of the *C.frutescens* were collected from the Raitu Bazaar, Raichur, India in the month of July and were authenticated and confirmed by the botanist of V.L. College of Pharmacy, Raichur, Karnataka. Then it was dried in shade at room temperature and subjected to size reduction to a fine powder using grinder mixer.

Preparation of aqueous extract

The fruit powder of *C.frutescens* was macerated with distilled water (containing 2% chloroform (10 ml), which acts as preservative) for 24 h with occasional stirring for every 60 min. Then the resultant was filtered through muslin cloth. The filtrate was dried on a water bath maintained at 45 °C to get a solid mass. All these extracts were stored in an airtight container in a refrigerator below 10 °C.

Experimental animals

Wister strain of albino rats of both sex (150–200 g) and either sex of albino mice (16–25 g) were collected from the National Centre for Laboratory Animal Science, Bengaluru, India to carry out the experimental study. All the animals were acclimatized for one week using standard animal husbandry conditions, as per CPCSEA guideline. Synthetic standard diet food (From Pranava Agro Industries ltd, Sangli, India) was supplied to all animals. The study protocol was approved by Institutional animal ethical committee (IAEC) with a registration no. 557/08/c/CPCSEA.

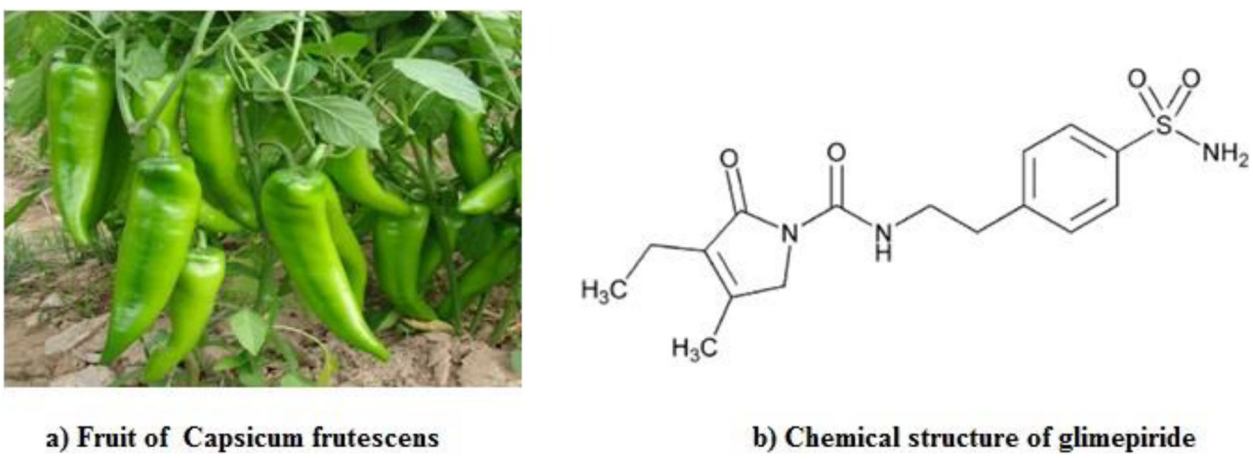


Fig. 1 Image of *C.frutescens* and chemical structure of glimpiride

Chemicals and drugs

Glimepiride was obtained from the Dr. Reddys Lab, Hyderabad, India. Glucose kit from Erba Mannheim, Mumbai, India, Trisodium citrate and Citric acid monohydrate form S.d.fine chemicals limited, Bengaluru, India.

Determination of acute toxicity (LD_{50})

The oral acute toxicity study [9] of the extract was determined by using albino mice of either sex (16–25 g), maintained under standard husbandry conditions. The animals were fasted for 3 h prior to the experiment. Animals were administered with single dose of extract of *C.frutescens* and observed for its mortality up to 48 h study period (short term toxicity). Based on the short-term toxicity profile, the next dose was determined as per OECD Guidelines No 425. From the LD_{50} dose 1/20th, 1/10th and 1/5 th doses were selected and considered as low, medium and high dose respectively and used in the entire study.

Induction of diabetes

Rats of either sex weighing between 175 and 200 g were selected and fasted for 18 hs prior to experiment and water supplied ad-libitum. The animals were kept in colony cages at ambient temperature of 28 ± 2 °C and relative humidity 45 to 55% with a 12 h light/ dark cycle. The rats were administered with 45 mg/Kg of Streptozotocin intraperitoneally [10].

Dose selection of glimepiride

The human dose (4 mg/Kg, p.o.) of Glimepiride was extrapolated to rat dose based on body surface area and weight. The dose effect relationship is established using ½ TD, TD and 2 TD (i.e; TD is therapeutic dose) the human dose (4 mg/Kg, p.o.) of Glimepiride extended to rat as ½ TD (0.036 mg/200 g, p.o.), TD (0.072 mg/200 g, p.o.) and 2 TD (0.144 mg/ 200 g, p.o.) [11]

Method for oral administration of drug

An 18 –gauge needle was suitably covered with flexible polythene tubing, where the edge was made blunt; the needle was fixed to 1 ml tuberculin syringe. The rat was held firmly in left hand, the tubing was moistened with glycerine and inserted right into the esophagus and gently pressing plunger for drug administration, and this was followed by 0.2 ml of distilled water to ensure administration of correct dose of the drug.

Method for collection of blood sample

The rat was placed into the rat holder, such that the tail was pulled out and was deplitated for collection of blood sample [12]. Tail vein was dilated by focusing a low voltage electric lamp. The tip of the tail was thin sliced (0.05 mm) using a sharp scissors. The blood drops were collected though the walls of 0.5 ml of centrifuge tube (to avoid haemolysis of the blood sample). The tail was gently pressed with fingers to enhance the blood flow and allowed to clot in centrifuge tube. Later dry cotton was applied for few minutes to stop the blood flow and the tail was sterilized by spirit.

Method of collection of serum

The serum was obtained by centrifuging the blood samples for 20 min (3000 rpm), supernatant fluid was decanted into the clean dry test tube.

Experimental study in diabetic rats

The groups of Wistar albino rats (35) of either sex weighing 160–180 g were selected for the study and kept in colony cages at ambient temperature of 28 ± 2 °C and relative humidity of 45 to55% with a 12 h light/dark cycle. The animals were fasted for 18 h before commencing the experiment with water ad libitum. The fasting was continued till completion of the experiment. The '0' h blood samples were collected for estimation of fasting serum glucose.

In stage 1: anti diabetic activity one day study

The study is planned to assess the hypoglycemic activity of single doses of AQEFCF and glimepiride in diabetic rats. Group 1 animals are administered wih 0.1 N NaOH vehicle, Groups 2, 3 and 4 were administered with 100, 200 and 400 mg/Kg AQEFCF and Group 5, 6 and 7 are administered with 1/2TD, TD and 2 TD glimepiride.

In stage 2: anti diabetic activity one week study

The present study is planned to assess the hypoglycemic activity of repeated doses of AQEFCF and glimepiride in diabetic rats. Group 8 was administered with 0.1 N NaOH vehicle Groups 9, 10 and 11 are administered with 100, 200 and 400 mg/Kg AQEFCF and Groups 12, 13 and 14 are administered with 1/2TD, TD and 2 TD glimepiride.

In stage 3: food-drug interaction one day study

The study was planned to evaluate the influence of single dose of AQEFCF on hypoglycemic activity of single dose of glimepiride in diabetic rats. In this, Group 15 was given AQEFCF 100 mg/Kg + glimepiride ½ TD, Group 16 received AQEFCF 200 mg/Kg + glimepiride ½ TD Group 17 received AQEFCF 400 mg/Kg + glimepiride ½ TD Group 18 received AQEFCF 100 mg/Kg + glimepiride TD Group 19 received AQEFCF 200 mg/Kg + glimepiride TD Group 20 received AQEFCF 400 mg/Kg + glimepiride TD Group 21 received AQEFCF 100 mg/Kg + glimepiride 2 TD, Group 22 received AQEFCF 200 mg/Kg + glimepiride 2 TD and Group 23 received AQEFCF 400 mg/Kg + glimepiride 2 TD.

In stage 4: food-drug interaction 8 days study

The interaction study was planned to investigate the effect of repeated doses of AQEFCF for 8 days followed by glimepiride on 8th day in diabetic rats. In this, Group 24 received AQEFCF 100 mg/Kg + glimepiride ½ TD Group 25 received AQEFCF 200 mg/Kg + glimepiride ½ TD Group 26 received AQEFCF 400 mg/Kg + glimepiride ½ TD Group 27 received AQEFCF 100 mg/Kg + glimepiride TD Group 28 received AQEFCF 200 mg/Kg + glimepiride TD Group 29 received AQEFCF 400 mg/Kg + glimepiride TD Group 30 received AQEFCF 100 mg/Kg + glimepiride 2TD Group 31 received AQEFCF 200 mg/Kg + glimepiride 2TD and Group 32 received AQEF CF 400 mg/Kg + glimepiride 2TD. Animals are administered with AQEFCF for one week. Later, the rats are fasted for 18 h. On the 8th day, after collecting the fasting blood samples from tail vein AQEFCF is given orally to all the rats and 30 min later, glimepiride is administered orally. Serum glucose levels are estimated.

In stage 5: food-drug interaction 8 days study (selective doses from stage 4)

The study was planned to evaluate food-drug interaction between repeated doses of both AQEFCF and glimepiride for 8 days in diabetic rats. In this Group 33 received AQEFCF 400 mg/Kg + glimepiride ½ TD Group 34 received AQEFCF 400 mg/Kg + glimepiride TD Group 35 received AQEFCF 400 mg/Kg + glimepiride 2TD. Animals are administered with AQEFCF and glimepiride with an interval of 30 min for one week. Later, the rats are fasted for 18 h. On the 8th day, after collecting the fasting blood samples from tail vein AQEFCF is given orally to all the rats and 30 min later, glimepiride is administered orally. Serum glucose levels are estimated.

Percentage reduction in serum glucose at time "t" t = A-B/A × 100

Where:

A is serum glucose concentration at time "0" and

B is serum glucose concentration at time "t"

Chemiluminescence Immunoassay Method for Insulin

To carry out the assay of control and test in duplicate, all the micro plate wells were formatted. Replace any unused micro well strips back into the aluminum bag, seal and store at 2–8 °C.Pipette 0.05 ml (50 μl) of the appropriate calibrators, controls and samples into the assigned wells. Add 0.1 ml of the insulin tracer reagent to each well. For the proper mixing micro plate swirl gently for 20–30 s. Plastic wrap is used to cover the plates and incubate at room temperature for 60 min. Read the Relative light units using a 96 well micro plate laminator for 0.2–1.0 s per well. The results should be read within 30 min of adding the stop solution. The microplate contents were discarded by aspiration or decantation. For decanting tap and blot the microplates with the use of dry adsorbent paper. Wash buffer (350 μL) was used to decant or aspirate. Repeat the procedure for five washed. Manual or automatic plate washer can also be used. Add working signal reagent (0.1 mL) to all the wells. In the same order the reagents were added to minimize reaction time difference between the wells and incubate in the dark for 5 min at room temperature [13].

Statistical analysis

The results were expressed as the mean ± SEM and were analyzed by one-way ANOVA followed by Dunnett's multiple comparison "t" test. Data was computed for statistical analysis by using Graph Pad PRISM 5 Software.

Pharmacokinetic study

In this study 24 Wister albino rats of either sex (160–180 g) were randomly selected and divided in to four groups containing six rats in each group. Animas were kept fasting for 18 h except water. For this study the single dose if glimepiride 1TD (0.72 mg/200 mg) and single dose of *C. frutescens* (200 mg/kg) was administered. The drugs were administered through the oral route using oral feeding needle (size --) to various groups in following order.

Group I: Control

Group II: Glimepiride (0.072 mg/200 g)

Group III: 200 mg/kg C.frutescens

Group IV: 200 mg/kg C.frutescens for 14th day. On 15th day *C. frutescens* 200 mg/kg, followed by glimepiride 0.072 mg/200 g

The blood samples (0.6 mL) were collected through the retro-orbital route, with light anaesthesia using 0.2% Phenobarbital sodium in i.p route. The blood samples were collected at the time internal of 0, 0.5, 1, 1.5, 2, 2.5, 4, 6, 8,10, 12 and 24 h of administered dose. The collected blood samples were transferred to polypropylene tube (K2 EDTA, J. K diagnostic, Rajkot). The plasma was separated immediately and processed to obtain the plasma samples by protein precipitation method, that has been described in the section of "plasma extraction method." The plasma samples were then injected in to the chromatographic system. The obtain data were used to construct a plasma concentration vs time profile. The pharmacokinetic parameters were obtained using linear log trapezoidal rule method.

Plasma extraction method

The blood samples that has been withdrawn were processed by protein precipitation technique [14]. Accurately 0.2 mL of blood sample were transferred in to a micro centrifuge tube and spiked with 20 μL of 20 ng/mL of aqueous internal

standard (glipizide) solution. 0.5 mL of acetonitrile was added to the tube. The samples were vortexed for 30 s to ensure complete mixing. Separation of two phase were performed by centrifugation at 7000 rotation per minute for the time period of 5 min. The supernatant was transferred in to other glass tube followed by complete evaporation under a stream of nitrogen at 40 °C. The obtained dry residue was reconstituted with 120 μL mobile phase. Finally 20 μL of above sample was injected in to the chromatographic system.

Optimised analytical condition

Plasma glimepiride was determined using a validated LC-MS/MS method [15]. The separation was achieved on Zorbax C-18 stable band (4.6 mm id × 50 mm) column. The tendem mass spectroscopy was performed with API 3000 triple quadrupole mass spectrophotometer (ABSCIEX, Foster city, Ontario, Canada.). The mobile phase consist of 80% acetonitrile and 20% deionised water (pH 3.5 adjusted with acetic acid) used after diagnosis, with a flow rate of 200 μL/min and 2.5 min run time. Glimepride and internal standard (glipizide) were detected by MRM scan with positive ion mode. The tuning parameters consist of 20v cone voltage, extractor at 2v, source temperature at 120 °C, collision cell entrance potential at − 1.0 V, with a collision energy 12 eV and dwell time of 0.5 s.

Results

Acute oral toxicity study

When the aqueous extract of C.frutescens (AQEFCF) was administered orally to different groups of mice at different dose levels, it was found that even up to the dose level of 2000 mg/Kg body weight, the extract showed no effect either on behavioral symptoms or mortality during the observation period of 48 h (short term toxicity) and no mortality observed up to 14 days of experimental study (long term toxicity).

Anti diabetic activity of AQEFCF and glimepiride

0.1 N NaOH used as vehicle in control has not shown any reduction in the serum glucose levels in both single and in repeated treatment studies

Effect of single dose treatment of AQEFCF on serum glucose levels in diabetic rats

AQEFCF with three different doses like 100, 200, 400 mg/Kg p.o. has produced a dose dependent reduction in serum glucose levels in diabetic rats. The maximum serum glucose reduction observed at 6th h of each individual dose recorded as 15.32%, 19.63% and 22.16% respectively was shown in Table 1.

Effect of single dose treatment of glimepiride on serum glucose levels in diabetic rats

Glimepiride with three different doses like ½ TD, TD, and 2TD has produced a dose dependent reduction in serum glucose levels in diabetic rats. The maximum serum glucose reduction was observed at 6th h of each individual dose recorded as 33.10%, 39.79% and 48.67% respectively was shown in Table 2.

Effect of repeated dose treatment of AQEFCF on serum glucose levels in diabetic rats

AQEFCF with three different doses like 100, 200, 400 mg/Kg p.o. has produced a dose dependent reduction in serum glucose levels in diabetic rats. The maximum serum glucose reduction observed with these three doses is 17.19%, 24.93 and 30.76 all at 6th h respectively.

Effect of repeated dose treatment of glimepiride on serum glucose levels in diabetic rats

Glimepiride with three different doses like ½ TD, TD, 2 TD p.o. has produced a dose dependent reduction in serum glucose levels in diabetic rats. The maximum serum glucose reduction observed with these three doses is 40.39, 48.61, 53.76% respectively and all noted at 6th h. When compared with vehicle control, AQEFCF (100, 200, 400 mg/Kg p.o.) single and repeated treatment groups has shown a dose dependent and significant reduction in serum glucose levels throughout the experimental study. When compared with vehicle control glimepiride (1/2TD, TD, and 2 TD p.o.) single and repeated treatment groups has shown a dose dependent and significant reduction in serum glucose levels throughout the experimental study. All the treated groups i.e. AQEFCF 100, 200, 400 mg/Kg p.o. and glimepiride ½TD, TD, 2TD p.o. has shown a maximum reduction of 15.32, 19.63, 22.16% and 33.10, 43.85, 50.55% after single doses treatment and 17.19, 24.93, 30.76% and 40.39,48.61, 53.76% in repeated doses treatment respectively at 6th h .

Influence of single dose treatment of AQEFCF on antidiabetic activity of glimepiride (single dose) in diabetic rats (one day interaction study)

AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride ½ TD (0.036 mg/200 g, p.o) in each group has produced a significant reduction in serum glucose levels in diabetic rats. The maximum serum glucose reduction observed with these three doses is 33.59%, 34.04 and 35.22% respectively at 6th h. AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride TD (0. 072 mg/200 g, p.o) has produced a significant reduction in serum glucose levels in diabetic rats. The maximum serum glucose reduction observed with these three doses is 40.21%, 41.82% and 42.12% respectively at 6th h. AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride 2 TD (0. 144 mg/200 g, p.o) has produced

Table 1 Effect of single dose of *C. frutescens* on serum glucose level and % reduction in diabetic rats

Time (hr)	SERUM GLUCOSE (mg/dL)			% REDUCTION		
	C. frutescens (100 mg/kg)	*C. frutescens* (200 mg/kg)	*C. frutescens* (400 mg/kg)	*C. frutescens* (100 mg/kg)	*C. frutescens* (200 mg/kg)	*C. frutescens* (400 mg/kg)
0	309.17 ± 2.08	311.62 ± 2.09	307.38 ± 1.56	–	–	–
1	296.72 ± 3.59	296.20 ± 1.91	288.20 ± 1.85	4.02 ± 1.08**	4.94 ± 0.77***	6.24 ± 0.64***
2	286.95 ± 3.15	285.38 ± 1.57	281.23 ± 1.17	7.18 ± 0.99***	8.41 ± 0.79***	8.50 ± 0.12**
3	277.33 ± 3.00	272.32 ± 2.83	266.33 ± 1.24	10.28 ± 1.15***	12.60 ± 1.11***	13.35 ± 0.61***
4	269.80 ± 2.95	259.18 ± 3.05	254.53 ± 2.72	12.71 ± 1.21***	16.80 ± 1.32***	17.17 ± 1.00***
6	261.72 ± 2.79	250.35 ± 2.91	239.25 ± 5.00	15.32 ± 1.15***	19.63 ± 1.23***	22.16 ± 1.65***
8	265.40 ± 2.75	258.77 ± 3.01	250.02 ± 4.16	14.14 ± 0.96***	16.93 ± 1.31***	18.67 ± 1.19***
10	272.43 ± 2.74	267.42 ± 3.44	262.35 ± 2.63	11.86 ± 1.01***	14.47 ± 1.48***	14.65 ± 0.82***
12	285.72 ± 1.90	275.80 ± 3.23	270.42 ± 2.00	7.57 ± 0.69***	11.47 ± 1.44***	12.02 ± 0.50***
16	293.28 ± 1.55	286.47 ± 1.60	280.15 ± 2.61	5.13 ± 0.57***	8.06 ± 0.49***	8.83 ± 1.20**
24	301.84 ± 2.22	298.70 ± 2.59	292.23 ± 1.76	2.37 ± 0.40*	4.14 ± 0.54***	4.91 ± 0.75*

n = 6, ns- non significant, significant at *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ when compared to control group

a significant reduction in serum glucose levels in diabetic rats. The maximum serum glucose reduction observed with these three doses is 51.47%, 51.95% and 52.95% respectively at 6th h shown in Fig. 2 and Table 2.

Influence of repeated dose treatment of AQEFCF on antidiabetic activity of glimepiride (single dose) in diabetic rats (8 days study)

AQEFCF 100, 200, 400 mg/Kg, p.o. repeated dose treatment followed by glimepiride ½ TD (0.036 mg/200 g, p.o) has produced a significant reduction in serum glucose levels in diabetic rats. The maximum serum glucose reduction observed with these three doses is 35.12%, 36.07% and 37.35% at respectively 6th h. AQEFCF 100, 200, 400 mg/Kg, p.o. repeated dose treatment followed by glimepiride TD (0. 072 mg/200 g, p.o) has produced a significant reduction in serum glucose levels in diabetic rats. The maximum serum glucose reduction observed with these three doses is 40.40%, 43.64% and 46.44% respectively at 6th h. AQEFCF 100, 200, 400 mg/Kg, p.o. repeated dose treatment followed by glimepiride 2 TD (0. 144 mg/200 g, p.o) has produced a significant reduction in serum glucose levels in diabetic rats. The maximum serum glucose reduction observed with these three doses is 51.62%, 52.51% and 53.27% respectively at 6th h shown in Fig. 3 and Table 3.

Influence of repeated dose treatment of AQEFCF on antidiabetic activity of glimepiride (repeated dose treatment) in diabetic rats (8 days study)

AQEFCF 400 mg/Kg repeated dose treatment along with repeated doses of ½ TD, TD and 2TD respectively has produced a significant reduction in serum glucose levels. The maximum reduction observed with the above treatment is recorded as 43.91%, 49.09 and 57.60% all at 6th h respectively. The results are shown in Table 4.

Table 2 Antidiabetic activity of Glimepiride (GLIM) in diabetic rats

Time (hr)	SERUM GLUCOSE (mg/dL)			% REDUCTION		
	½ TD GLIM	1TD GLIM	2 TD GLIM	½ TD GLIM	1TD GLIM	2 TD GLIM
0	307.47 ± 2.89	304.98 ± 6.80	306.15 ± 5.12	–	–	–
1	291.43 ± 4.13	287.53 ± 6.70	270.89 ± 3.23	5.18 ± 1.57***	5.69 ± 1.23***	11.31 ± 2.48***
2	266.73 ± 3.51	261.10 ± 6.42	250.87 ± 5.80	13.24 ± 0.92***	14.37 ± 1.26***	18.06 ± 1.30***
3	241.17 ± 4.12	234.15 ± 3.69	223.65 ± 5.50	21.52 ± 1.61***	23.14 ± 1.10***	26.90 ± 1.76***
4	224.82 ± 2.94	200.07 ± 5.61	188.58 ± 6.10	26.87 ± 0.79***	34.37 ± 1.14***	38.43 ± 1.48***
6	205.67 ± 1.54	183.53 ± 3.91	157.18 ± 4.71	33.10 ± 0.45***	39.79 ± 0.76***	48.67 ± 1.20***
8	222.82 ± 5.28	189.47 ± 3.21	181.50 ± 3.76	27.55 ± 1.36***	37.75 ± 1.51***	40.66 ± 1.33***
10	229.10 ± 4.15	206.02 ± 5.50	201.92 ± 4.51	25.50 ± 0.97***	32.36 ± 1.82***	34.03 ± 1.11***
12	252.68 ± 4.37	235.18 ± 6.49	224.60 ± 4.47	17.80 ± 1.35***	22.86 ± 1.53***	26.57 ± 1.58***
16	269.00 ± 3.21	258.45 ± 6.56	241.93 ± 1.44	12.51 ± 0.72***	15.26 ± 1.08***	20.86 ± 1.43***
24	284.13 ± 4.78	274.15 ± 5.75	261.98 ± 7.00	7.60 ± 1.12***	10.04 ± 1.47***	14.46 ± 1.41***

n = 6, ns- non significant, significant at *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ when compared to control group

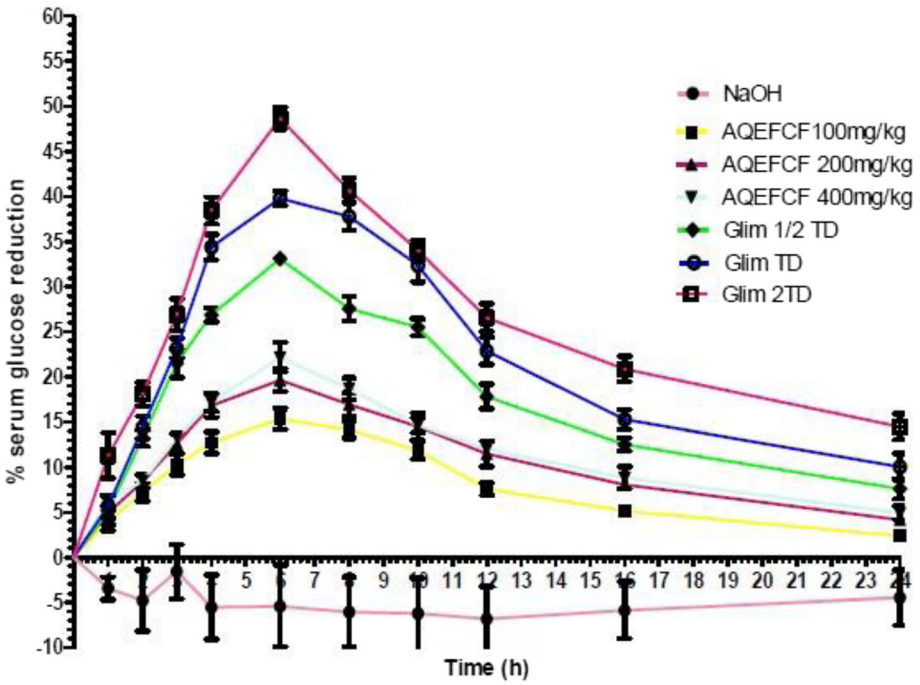


Fig. 2 Effect of single dose of *C.frutescens* and glimepiride on serum glucose level in diabetic rats

Influence of single dose treatment of AQEFCF on antidiabetic activity of glimepiride (single dose) in diabetic rats (one day interaction study)

When compared with single dose of glimepiride ½ TD treated group AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride ½ TD (0.036 mg/ 200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at3, 8, 10, 12, 16 and 24 h with 100 mg, 3, 4, 6 and 12 h with 200 mg and 3, 6 and 12 h with 400 mg.

AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride TD (0. 072 mg/200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 2, 4, 6, 8, 10, 12, 16 and 24 h except 24 h

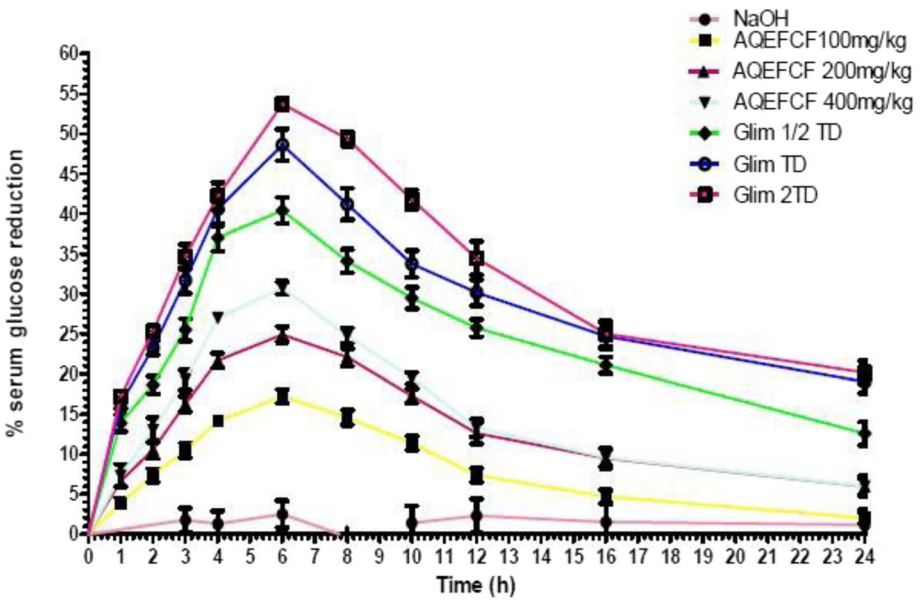


Fig. 3 Effect of repeated dose of *C.frutescens* and glimepiride on serum glucose level in diabetic rats

in 100 mg/Kg treated group. AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride 2 TD (0. 144 mg/ 200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at all time intervals of the experimental study.

When compared with single dose of glimepiride TD treated group AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride ½ TD (0.036 mg/ 200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 4, 6, 8, 10 h except 24 h in 400 mg/Kg treated group (Table No. 29, 30, 31).AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride TD (0. 072 mg/200 g, p.o) in respective groups has not shown a significant reduction in blood glucose level.. AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride 2TD (0. 144 mg/200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at1, 2, 4, 6, 8, 10, 12, 16, 24 h except 10th h in 200 mg/Kg treated group. When compared with single dose of glimepiride 2TD treated group AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride ½ TD (0.036 mg/200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 3, 4, 6, 8, 10, 12, 16, 24 h except 3, 12, 24 h in 400 mg/Kg treated group .

AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride TD (0. 072 mg/200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 4, 6, 16 h; 3, 4, 6 h and 4, 6 h respectively. AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride 2TD (0. 144 mg/200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 8 h with 100 mg/Kg dose but not significant with 200 mg/Kg at any time interval and 4, 6, 10 h with 400 mg/Kg dose .

Influence of repeated dose treatment of AQEFCF on glimepiride (single dose) in diabetic rats (8 days study)

When compared with single dose of glimepiride ½ TD treated group Repeated dose of AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride ½ TD (0.036 mg/ 200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 1 and 3 h with 200 and 400 mg/Kg doses but has not shown any a significant reduction with 100 mg/Kg dose. Repeated dose of AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride TD (0. 072 mg/ 200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 1, 2, 4, 6, 8, 10, 12, 16, 24 h with 200 mg and 400 mg/Kg doses but not significant at 1, 12 and 24 h with 100 mg/Kg dose. Repeated dose of AQEF CF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride 2TD (0. 144 mg/ 200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24 h except 24 h with 400 mg/Kg dose.

When compared with single dose of glimepiride TD treated group Repeated dose of AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride ½ TD (0.036 mg/ 200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 4, 6 and 8 h except 6th h with 200 mg and 400 mg/Kg doses. Repeated dose of AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride TD (0. 072 mg/ 200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 1, 3, 6 and 10 h except 1 and 3 h with 100 mg/Kg and 3 and 6 h with 200 mg/Kg doses. (Table No. 41, 42, 43). Repeated dose of AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride 2TD (0. 144 mg/200 g, p.o) in respective groups has

Table 3 Influence of single dose treatment of *C. frutescens* (400 mg/kg) on antidiabetic activity of glimepiride in diabetic rats

Time (hr)	SERUM GLUCOSE (mg/dL)			% REDUCTION		
	400 mg *C. frutescens*			400 mg *C. frutescens*		
	½ TD GLIM	1TD GLIM	2 TD GLIM	½ TD GLIM	1TD GLIM	2 TD GLIM
0	322.05 ± 2.45	316.25 ± 2.67	305.40 ± 4.41	–	–	–
1	295.33 ± 3.89	286.65 ± 3.35	266.70 ± 2.70	8.29 ± 1.15^{ns}	9.36 ± 0.79 ns	$15.89 \pm 1.92^{***\#\#\#}$
2	272.58 ± 5.15	258.08 ± 2.74	241.87 ± 3.51	15.36 ± 1.50^{ns}	18.38 ± 0.77*	$20.65 \pm 2.28^{***\#\#}$
3	248.97 ± 3.85	235.38 ± 5.46	220.38 ± 3.34	22.69 ± 1.07^{ns}	25.61 ± 1.16^{ns}	$27.07 \pm 1.88^{***\#\#}$
4	224.78 ± 3.12	199.03 ± 2.68	180.37 ± 3.66	$30.19 \pm 1.00^{ns\$\$\$}$	$37.05 \pm 0.93^{***\$\$\$}$	$40.84 \pm 1.78^{***\#\#\$}$
6	28.63 ± 2.40	183.98 ± 2.36	143.42 ± 3.60	$35.22 \pm 0.58^{ns\#\$\$\$}$	$42.12 \pm 0.92^{***\$\$\$}$	$52.95 \pm 1.60^{***\#\#\#\$}$
8	224.95 ± 2.95	195.95 ± 5.09	165.58 ± 1.04	$30.13 \pm 1.10^{ns\#\#\$\$\$}$	$38.01 \pm 1.73^{***}$	$45.71 \pm 1.09^{***\#\#\#}$
10	235.97 ± 3.39	213.27 ± 3.85	189.88 ± 1.94	$26.67 \pm 1.55^{ns\#\$\$}$	$32.52 \pm 1.47^{**}$	$39.35 \pm 0.74^{***\#\#\$}$
12	250.0 ± 5.05	233.62 ± 3.90	213.70 ± 3.07	22.31 ± 2.01^{ns}	$26.10 \pm 1.42^{***}$	$31.72 \pm 1.35^{***\#}$
16	270.28 ± 4.84	252.57 ± 2.71	239.17 ± 3.13	$16.04 \pm 1.74^{ns\$\$\$}$	$20.12 \pm 0.94^{***}$	$23.61 \pm 1.16^{***\#\#\#}$
24	289.28 ± 2.65	262.98 ± 4.58	251.95 ± 4.03	10.18 ± 0.38^{ns}	$16.37 \pm 1.10^{***}$	$17.37 \pm 2.13^{***\#\#\#}$

n = 6, ns- non significant, significant at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to control group; $^{ns}P > 0.05$, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ when compared to glimepiride ½ TD, 1TD and 2 TD group; when compared to glimepiride TD group

Table 4 Influence of repeated dose treatment of *C. frutescens* (400 mg/kg) on antidiabetic activity of glimepiride in diabetic rats (8th day)

Time (hr)	SERUM GLUCOSE (mg/dL)			% REDUCTION		
	400 mg *C. frutescens*			400 mg *C. frutescens*		
	½ TD GLIM	1TD GLIM	2 TD GLIM	½ TD GLIM	1TD GLIM	2 TD GLIM
0	307.80 ± 2.09	293.47 ± 3.14	298.67 ± 3.20	–	–	–
1	272.80 ± 1.62	257.30 ± 2.69	249.77 ± 2.91	$11.35 \pm 0.91^{ns\#}$	$12.31 \pm 0.69^{***\#\#\#}$	16.33 ± 1.14***###
2	255.85 ± 1.85	235.50 ± 3.14	226.03 ± 4.52	16.88 ± 0.25^{ns}	$19.72 \pm 1.26^{*\#\#}$	24.28 ± 1.69***###$$$
3	234.20 ± 2.48	211.52 ± 4.35	207.52 ± 2.14	23.91 ± 0.36^{ns}	$27.94 \pm 1.03^{**\#}$	30.47 ± 1.15***###
4	202.45 ± 1.79	181.68 ± 4.30	173.92 ± 4.51	34.21 ± 0.80^{ns}	$38.10 \pm 1.22^{***}$	41.77 ± 1.14***###
6	192.78 ± 2.53	157.17 ± 4.85	139.60 ± 1.59	$37.35 \pm 0.91^{ns\$\$\$}$	$46.14 \pm 1.56^{***\#\#}$	53.27 ± 0.88***###
8	209.05 ± 2.29	173.96 ± 3.00	155.25 ± 3.17	$32.08 \pm 0.56^{ns\#\$\$\$}$	$40.71 \pm 1.07^{***\$\$}$	48.01 ± 1.00***###
10	222.03 ± 3.68	195.68 ± 1.99	183.33 ± 2.92	$27.88 \pm 0.83^{ns\$}$	$33.31 \pm 0.75^{***\#\#\#}$	38.61 ± 0.83***###
12	237.12 ± 4.45	211.05 ± 3.29	202.67 ± 3.10	22.98 ± 1.17^{ns}	$28.07 \pm 1.24^{***}$	32.13 ± 0.82***###$
16	255.87 ± 4.45	228.60 ± 2.26	232.05 ± 2.46	16.87 ± 1.17^{ns}	$22.08 \pm 0.92^{***\#\#}$	22.27 ± 1.00***##
24	269.80 ± 3.61	245.45 ± 3.27	243.35 ± 3.35	12.34 ± 1.07^{ns}	$16.99 \pm 0.90^{***\#\#}$	18.47 ± 1.23***###

n = 6, ns- non significant, significant at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to control group; $^{ns}P > 0.05$, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ when compared to glimepiride ½ TD, 1TD and 2 TD group; when compared to glimepiride TD group; $p < 0.05$, $p < 0.01$ and $$ p<0.001, ns = not significant when compared with glimepiride 2 TD

shown a significant reduction in blood glucose levels at 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24 h. When compared with single dose of glimepiride 2TD treated group Repeated dose of AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride ½ TD (0.036 mg/200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 1, 3, 4, 6, 8 and 10 h except 1 and 3 h with 200 mg and 1, 3 and 4 hwith 400 mg doses. Repeated dose of AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride TD (0. 072 mg/200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 6 and 8 h. Repeated dose of AQEFCF 100, 200, 400 mg/Kg, p.o. treatment followed by glimepiride 2TD (0. 144 mg/200 g, p.o) in respective groups has shown a significant reduction in blood glucose levels at 2 and 12 h but not observed at 12 h with 100 and 200 mg/Kg doses.

Influence of repeated dose treatment of AQEFCF on glimepiride (repeated dose) in diabetic rats (8 days study)

When compared with repeated dose ½ TD of glimepiride treated group AQEFCF 400 mg/Kg p.o. repeated dose treatment along with repeated doses of ½ TD, TD and 2TD respectively has not shown a significant reduction in serum glucose levels with ½ TD, significant reduction in glucose levels observed at 2, 3, 6, 8, 10, 12,16 and 24 h with TD and at all time intervals with 2 TD. When compared with repeated dose TD of glimepiride treated group AQEFCF 400 mg/Kg p.o. repeated dose treatment along with repeated doses of ½ TD, TD and 2TD respectively has produced a significant reduction in serum glucose levels at 3 and 8 h with ½ TD and 3, 6, 8, 10 and 12 h with 2TD and no significant reduction in serum glucose levels with TD. When compared with repeated dose 2TD of glimepiride treated group AQEFCF 400 mg/Kg p.o. repeated dose treatment along with repeated doses of ½ TD, TD and 2TD respectively has produced a significant reduction in serum glucose levels at 2, 3, 6, 8, 10 and 12 h with ½ TD and 6, 8 and 10 h with TD and no significant reduction in serum glucose levels with 2 TD.

Effect of a.vera and Glimepiride on serum insulin levels in diabetic rats by Chemiluminescence method

In one day interaction, all the combination groups shows significant ($P < 0.01$) increase in serum insulin levels when compared to diabetic control group. The maximum increase serum insulin levels observed with glimepiride 2TD after treatment of *C. frutescens* (400 mg/kg, p.o.) produced 13.15 μu/m. In 8 days interaction study, all the combination groups shows significant ($P < 0.01$) increase in serum insulin levels when compared to diabetic control group. The maximum increase serum insulin levels observed with repeated treatment of *C. frutescens* (400 mg/kg, p.o.) daily once for 8 days followed by administration with glimepiride 2 TD (0.144 mg/200 g, p.o.) 30 min later produced 21.15 μu/ml. as shown in Table 5.

Results of pharmacokinetic study

The obtained pharmacokinetic parameters in this present study were, area under the curve from 0 to last sampling time ($AUC_{0\text{-}t}$) with a value of 1272 (ng/mL.hr) for glimepiride alone, 1258 (ng/mL.hr) for 1st day

Table 5 Serum insulin levels on Single and repeated dose administration of *C. frutescens* and glimepiride in diabetic rats (chemi-luminescent immune assay method)

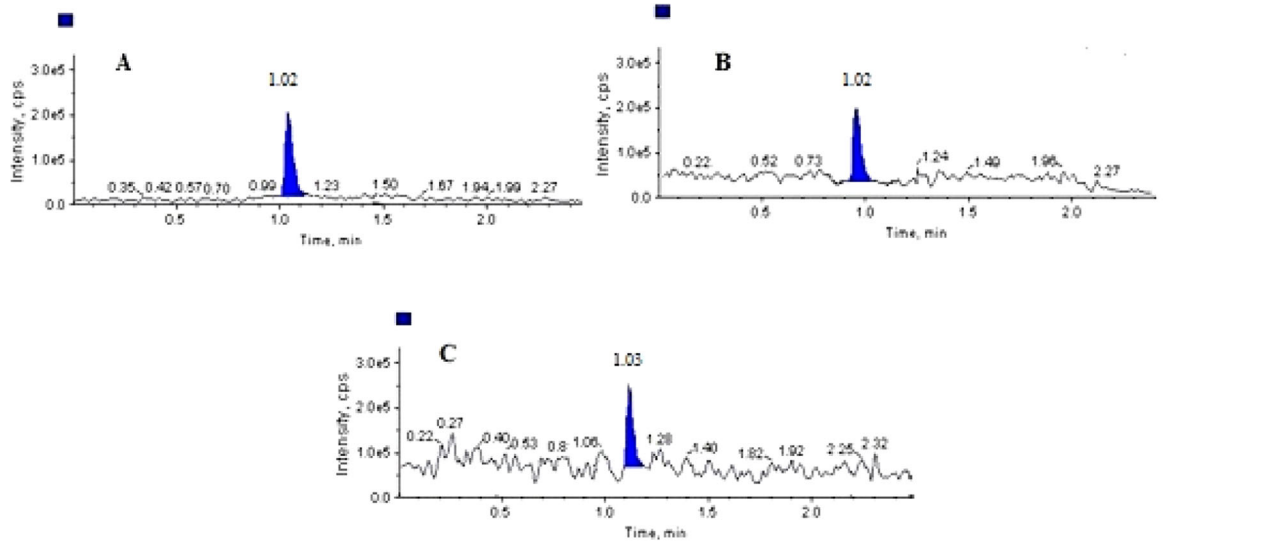
Groups	1st day Mean ± SEM	8th day Mean ± SEM
C. frutescens 400 mg/kg + ½ TD GLIM	9.62 ± 0.11**	13.37 ± 0.78**
C. frutescens 400 mg/kg + 1TD GLIM	10.86 ± 0.51**	15.66 ± 0.3**
C. frutescens 400 mg/kg + 2 TD GLIM	13.15 ± 0.46**	21.15 ± 0.73**

n = 6, ns = not significant, significant at, $P < 0.01^{*}$, $P < 0.01^{**}$

interaction and 1238 (ng/mL.hr) for 15th day interaction. The T_{max} for glimepiride alone was 3.58 h, 3.58 h for 1st day interaction and 3.43 h for 15th day interaction. MRM (multiple reaction monitoring) chromatograms from rat plasma samples during pharmacokinetic study was shown in Fig. 4. All other parameters has been summarised in the Table 6 and plasma concentration and time was shown in Fig. 5.

Discussion

Diabetes, which is metabolic disorder, results may be due to insulin resistance or glucose transport inability from blood to cells [16]. Glimepiride, an oral hypoglycemic drug of the sulfonylurea class indicated to lower the blood glucose in patients with noninsulin-dependent (Type-2) diabetes mellitus (NIDDM). The primary mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning islets of langerhans of pancreatic β- cells. In addition, an extra pancreatic effect can also leads to increased sensitivity of peripheral tissues to insulin [17]. The literature reveals that *C.frutescens* strengthens the heart and cleanses the lymphatic system and bowel and also stimulates the kidneys. It act as a tonic for the whole body and is used in conditions such as arthritis, rheumatism, fibromyalgia, cramps, cancer, overweight, stomach aches, gas bloating, yellow fever, fever and for gangrene. It is also used with success against angina, alcoholism, asthma, atherosclerosis, bleeding gums, blood clots, bowel diseases bruises, diabetes, diabetic neuropathy, duodenal ulcers, hyper triglyceremia, fatigue, food poisoning, disease conditions due to free radical activity, frost bite, frozen limbs, hardening of the arteries, arrhythmias, haemorrhages, hypertension, inflammation, impotence, indigestion, influenza, itching, joint pain, laryngitis, lumbago, menstrual cramping, motion sickness, mouth sores, multiple sclerosis, muscle aches, inflammation of nerve, neuralgia, night blindness, obesity, pain, peptic ulcer, poor appetite, psoriasis, respiratory disorders, sea sickness, shingles, sores, stomach ulcers, tooth ache, wound, *Herpes zoster* and shingles. An increasing number of medicinal plants are



A. MRM chromatogram of Glimepiride ***at 4th hour of blood sample collection***

B: MRM chromatogram of Glimepiride with ***C. frutescens (1st day interaction)at 4th hour of blood sample collection***

C: MRM chromatogram of Glimepiride with ***C. frutescens (15th day interaction)at 4th hour of blood sample collection***

Fig. 4 MRM chromatogram of glimepiride form extracted rat plasma samples at 4th hour alone (**a**), glimepiride with *C. frutescens* 1st day interaction (**b**), glimepiride with *C. frutescens* 15th day interaction (**c**)

Table. 6 Various pharmacokinetic parameters

Parameters	Glimepiride	Glipmepiride + *C. frutescens* (1st day interaction)	Glipmepiride + *C. frutescens* (15th day interaction)
C_{max} ng/mL	71.23 ± 23.22	70.98 ± 22.34	71.03 ± 36.33
T_{max} hours	3.6 ± 1.27	3.58 ± 0.98	3.43 ± 1.11
$T_{1/2}$ h	6.93 ± 2.10	6.81 ± 1.29	6.78 ± 2.32
$AUC_{0\text{-}t}$ (ng/mL/hr)	1272 ± 34.45	1258 ± 46.79	1238 ± 41.85
$AUC_{0\text{-}\infty}$ (ng/mL/hr)	162.62 ± 44.56	160.12 ± 21.65	161.32 ± 43.67

being used to treat diabetes and its related conditions. Many of these plants have been used ethnopharmacologically in traditional medicine as antidiabetics, particularly for T2DM. Te co-administration of antidiabetic herbs and pharmaceutical agents may result in enhanced efects (which may be desirable clinically), decreased pharmacological efects, or adverse drug events, such hypoglycemia. Combination with ginger extract reduces blood glucose level greater than Glimiperide alone. The present study was undertaken to evaluate any possible food-drug interaction between *C.frutescens* and glimepiride with single and repeated dose treatment of *C.frutescens* on hypoglycemic and antidiabetic activity of glimepiride in normal and diabetic rats. When the aqueous extract of *C.frutescens* (AQEFCF) was administered orally to different groups of mice at different dose levels, it was found that even up to the dose level of 2000 mg/Kg body weight, the extract showed no effect either on behavioral symptoms or mortality during the observation period of 48 h (short term toxicity) and no mortality observed up to 14 days of experimental study (long term toxicity). 0.1 N NaOH used as vehicle in control has not shown any reduction in the serum glucose levels in both single and in repeated treatment studies. Based on the results it can concluded that, ½ TD, TD and 2 TD glimepiride doses exhibited a significant and dose dependent antidiabetic activity. Similarly 100, 200, 400 mg/kg doses of AQEFCF also exhibited a significant and dose dependent reduction in serum glucose levels in diabetic rats. In one day interaction study 100, 200, 400 mg/kg doses of AQEFCF and ½ TD, TD and 2 TD glimepiride was increased the antidiabetic activity of 1%, 3% and 5% respectively. In 8 day interaction study /repeated dose treatment 100, 200 mg/kg doses of AQEFCF and single dose treatment of ½ TD, TD and 2 TD glimepiride was increased the antidiabetic activity of 2–4%, 1–

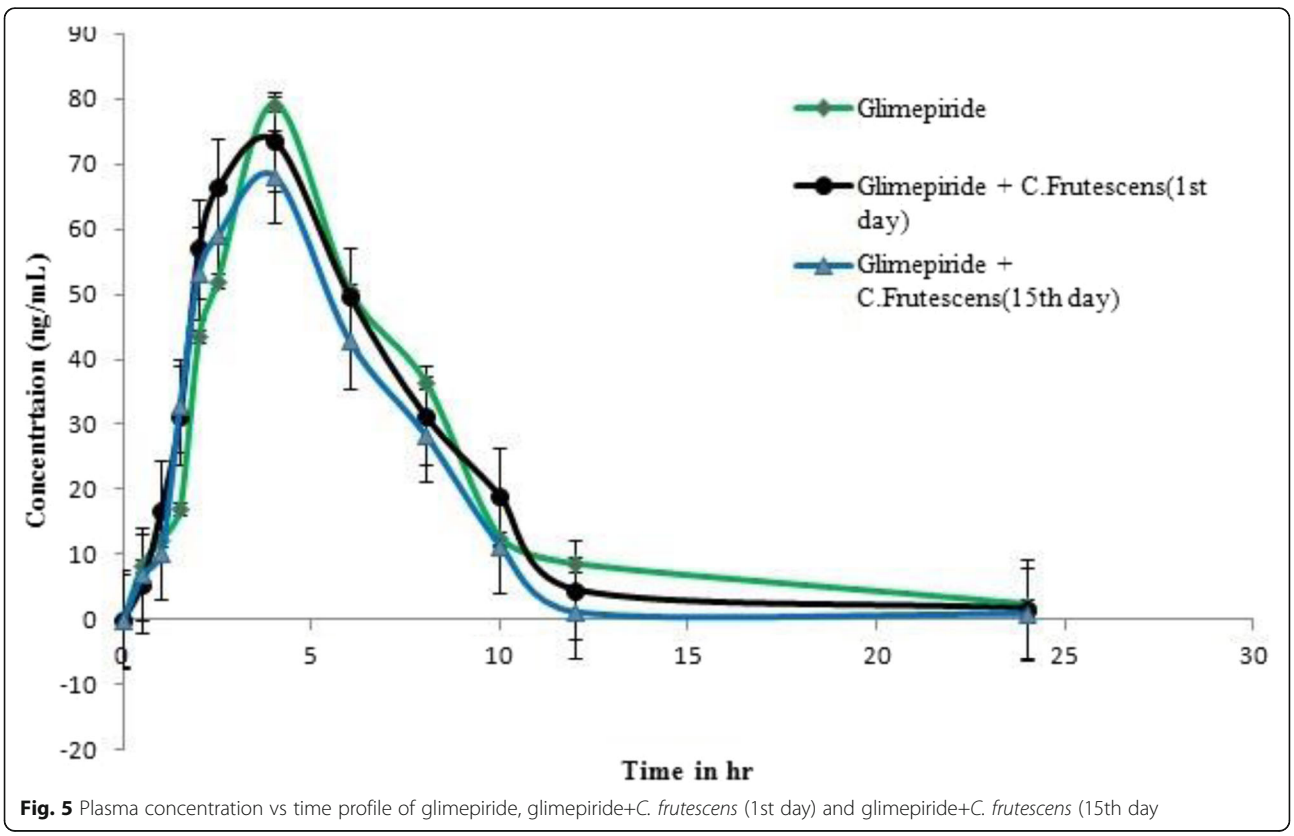


Fig. 5 Plasma concentration vs time profile of glimepiride, glimepiride+*C. frutescens* (1st day) and glimepiride+*C. frutescens* (15th day

7% and 3–5% respectively. Whereas 400 mg/kg of AQEF CF followed by single dose treatment of ½ TD, TD and 2 TD glimepiride was increased the antidiabetic activity of 3%, 1% and 4% respectively. In the present study results revealed that a significant increase serum insulin levels was observed in diabetic rats when treated with combination of *C.frutescens* and glimepiride when compared to single drug/herb treated groups. It is possible that *C.frutescens* may initiate cell proliferation, since it has been reported that pancreatic endocrine cells have the potential to proliferate after induction of diabetes with STZ (11). It was reported that glimepiride has effect on the β- cells and increase the insulin secretion (12). Therefore, the combination of *C.frutescens* (400 mg/kg) with glimepiride 2 TD (0.144 mg/200 g, p.o.) caused significant increase in serum insulin levels than individual treatment with glimepiride confirmed that extra increase in insulin levels are due to *C.frutescens* only. In the present study we were investigated the pharmacokinetic interaction of *Capsicum frutescens* L. and Glimepiride at therapeutic doses in healthy rats. There was no significant rise in plasma Glimiperide levels and pharmacokinetic parameters. In the 1st day and 15th day interaction study all the pharmacokinetic parameters values of only Glimiperide and Glimiperide with *Capsicum frutescens* L was found almost similar. Therefore it was stated that there was there is no pharmacokinetic interaction between *Capsicum frutescens* L. and Glimepiride.

Conclusion

Herb–drug interaction is an important issue affecting the efficacy and safety of therapeutic treatments. In the present study, no severe hypoglycemia or convulsions are noted with any of the experimental animals i.e., diabetic rats during study. Hence the study clearly confirmed that no significant food-drug interaction occurred between aqueous extract of *C.frutescens* and glimepiride either in combination of single doses or in combination of repeated doses because there was no influence on the pancreatic and extra pancreatic (cellular utilization of glucose) mechanisms that influence blood glucose.

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Authors' contributions
Dr. MA raja, Dr. P. Mondal designed the entire project work and carried acute toxicity study and herb- drug, interaction study in normal rats. Dr. MT, Mr. KV induced the diabetes to the animal and carried out herb- drug, interaction study in diabetic rats. Dr. Mondal and Mr. K Venu and P. Kiran has designed and carried out pharmacokinetic study. All the authors revised and approved the final manuscript and the research work was performed in collaboration between all the authors.

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Availability of data and materials
All data and material is available upon request.

Ethics approval and consent to participate
It is approved by the IAEC (Institutional animal ethical committee) of VL college of Pharmacy. Reference number of the ethics committee is 557/08/c/CPCSEA.

Consent for publication
Non Applicable.

Competing interests
The authors declare that they have no competing interest.

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