Herb–drug interaction study between *Aloe vera* and glimepiride in normal and diabetic rats

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Background
Herb–drug interaction study, which is the newest area of research, can affect the modern practice of medicine.
Aim
The present study was designed to explore the herb–drug interaction of *Aloe vera* gel, an herbal drug used in herbal formulation for hypoglycemic activity, with glimepiride in normal and diabetic rats.
Materials and methods
Lethal dose 50% studies for the aqueous extract of *A. vera* were carried out in albino mice up to the dose level of 2000 mg/kg by following 'up and down method' of Organization for Economic and Cooperation Development guidelines no. 425 of Committee for the Purpose of Control and Supervision of Experiments on Animals. Overall, 1/5th, 1/10th, and 1/20th doses of the maximum dose tested for lethal dose 50% of the aqueous extract were selected for the experimental study. *A. vera* (100, 200, and 400 mg/kg, postoperatively) and glimepiride ½ therapeutic dose (TD), 1 TD, and 2 TD (0.036, 0.072, and 0.144 mg/200 g, postoperatively) were administered orally alone as single doses and as well as concomitantly in normal and streptozotocin-induced diabetic rats.
Results
After the treatment in all the groups, serum glucose levels were determined, and the serum insulin levels were estimated only in the diabetic rats using chemiluminescence assay method. Both *A. vera* and glimepiride on their own when administered alone showed hypoglycemic effect in normal rats. The hypoglycemic effect observed with combination of *A. vera* and glimepiride was significantly more compared with either of the drugs given alone. *A. vera* also augmented the hypoglycemic effect of glimepiride in streptozotocin-induced diabetic rats.
Conclusion
Administration of *A. vera* with glimepiride increased the serum insulin levels. It has been concluded that *A. vera* augmented hypoglycemic effect of glimepiride. There was a herb–drug interaction that necessitates dose readjustment of glimepiride and monitoring of glucose levels to avoid hypoglycemic condition.

Keywords:
Aloe vera, diabetes mellitus, glimepiride, hypoglycemia, streptozotocin

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Introduction

Ayurvedic herbal preparations often consist of complex mixtures of plant materials, and Ayurveda is practiced in many countries of the Indian subcontinent [1]. Herbal products are used worldwide as home remedies in a variety of healthcare settings. These products are often promoted to the public as being 'natural' and completely 'safe' alternatives to conventional medicines. Nowadays, ~70–80% of population believes in the use of nonconventional medicine obtained from herbal resources, as has been reported by the WHO survey. Developed countries in the recent years have witnessed in the growth of the use of the over-the-counter medications in nutraceuticals and health fords [2]. Herbal medications generally include various dietary supplements, which contain either herb alone or in mixture form, known as botanicals. As herbal medicines are classified as dietary supplements, there are no Food and Drug Administration regulations regarding the accuracy of active ingredient content or efficacy and safety of active ingredients [3].

The herb–drug interaction study is among the newest areas of research, which has affected the modern

practice of medicine. Hence, information on specific interactions may simply not be available, as the research has not yet been conducted [4]. A herb–drug interaction can be defined as any pharmacological alteration caused by a herbal substance(s) to another chemical substances (e.g. a prescription medication) in the therapeutic, diagnostic, or other action of a drug in or on the body. This relates to the so-called drug–drug interactions (interactions between drugs), herb–herb interactions (interactions between herbs), or drug–food interactions (interactions between drugs and food). Herb–drug interaction is considered a type of drug interaction where the action of herb occurs by known or unknown chemical ingredient. Antidepressant herbs can cause a significant change in the cyclosporine A action in transplant patients. The plasma concentration can also decrease from a variety of drugs which include warfarin, theophylline, and digoxin. Herb–drug interaction sometimes may be beneficial, that is, by increasing the efficiency or decreasing adverse effect of an anticancer drug, for example, a clinical trial in Chinese herbal product recently demonstrated that the nausea induced by chemotherapy can be reduced.

However, many herb–drug interactions can also be harmful, for example, causing adverse reactions or therapeutic failure [5,6]. So the present study was planned to carry out the herb–drug interaction between *Aloe vera* gel, an ingredient of many herbal preparations, and glimepiride, a synthetic oral antidiabetic drug, as both possess hypoglycemic activity, in normal and diabetic rats.

Materials and methods

Plant material

The leaf of *A. vera* plant was collected in the month of June from Raichur district, formally identified and authenticated by Dr. B. Hementh Kumar, professor of pharmacognosy, with an authentication no. VLCP/plant-auth-31//2018/0321. The *A. vera* leaves were washed using warm water. The lower 1.5 inch of the leaf and the tapering 1–2 inch of the leaf top was taken for the gel collection. The spines were removed using a sharp knife. The mucilage layer, which presents below the green rind, has been collected.

Preparation of aqueous extract

Approximately 100 g of *A. vera* gel was taken into a round bottom flask (2000 ml) and macerated with 500 ml of distilled water and 10 ml of chloroform (preservative), and kept for 24 h with occasional shaking every hour. Then the marc was removed by filtering the extract, and then the extract was concentrated on a water bath maintained at 50°C. The extracts were examined for their color and consistency, and their percentage yield was calculated with reference to the quantity used for extraction. These two extracts were stored in airtight containers in a refrigerator below 10°C.

Experimental animals

Wistar strain albino rats of both sexes (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 1 week using standard animal husbandry conditions, as per Committee for the Purpose of Control and Supervision of Experiments on Animals guideline. Synthetic standard diet food (Pranava Agro Industries Ltd, Sangli, India) was supplied to all animals. The study protocol was approved by Institutional Animal Ethical Committee with a registration no. 557/02/c/Committee for the Purpose of Control and Supervision of Experiments on Animals.

Chemicals and drugs

Glimepiride (99.78% purity) was obtained as a gift sample from Dr. Reddy's Lab. (Bachupally, Hyderabad, India). Glucose kit was obtained from Erba Mannheim, Mumbai, and trisodium citrate and citric acid monohydrate were obtained from S. D. Fine Chem Limited, Bengaluru.

Determination of acute toxicity (lethal dose 50)

The acute toxicity [7] of *A. vera* was determined by using albino mice of either sex (18–23 g), maintained under standard husbandry conditions. Before the experiment, animals were fasted for 3 h. A single dose of root extract of *A. vera* was administered to the animals, and mortality was observed at a study period of 48 h (short-term toxicity). Based on the short-term toxicity profile, the next dose was determined as per Organization for Economic and Cooperation Development guidelines no. 425. From the lethal dose 50 dose, 1/20, 1/10, and 1/5th doses were selected and considered as low, medium, and high dose, respectively, to observe the dose-dependent action and used in the entire study.

Method for oral administration of drug

An 18-G 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 the left hand, the tubing was moistened with glycerin and inserted right into the

esophagus, and the plunger was gently pressed 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 depilated for collection of blood sample [8]. 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 hemolysis of the blood sample). The tail was gently pressed by the figures to collect sufficient blood and allowed to form a clot in a 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). The supernatant fluid was decanted into a clean dry test tube.

Herb–drug interaction study in normal rats

Glimepiride solution was prepared by dissolving in a small amount of 0.1 M NaOH solution and diluted to the desired volume with physiological saline solution. Distilled water was used to prepare *A. vera* solution. A total of 13 groups of Wistar albino rats of either sex weighing 180–200 g were selected for the study and were kept at ambient temperature of 28±2°C and relative humidity of 45–55% with a 12 h light/dark cycle. The animals were fasted for 18 h before commencing the experiment with water ad libitum. In this study, the normal rats of group 1 were administered with 0.1 M NaOH; groups 2, 3, and 4 were administered with 100, 200, and 400 mg/kg *A. vera*; groups 5, 6, and 7 were administered with ½ therapeutic dose (TD), 1 TD, and 2 TD glimepiride; group 8 was administered with *A. vera* 400 mg/kg+½ TD glimepiride; group 9 was administered with *A. vera* 400 mg/kg+1 TD glimepiride; group 10 was administered with *A. vera* 400 mg/kg+2 TD glimepiride to study the herb–drug interaction for 1 day; similarly, group 11 was administered with *A. vera* 400 mg/kg+½ TD glimepiride; group 12 was administered with *A. vera* 400 mg/kg+1 TD glimepiride; and group 13 was administered with *A. vera* 400 mg/kg+2 TD glimepiride to observe the herb–drug interaction for 8-day study, in which the dose of herb was repeated for 8 days followed by a single dose of drug (8th day of the study). The fasting was continued till completion of the experiment. The 0 h blood samples were collected for the estimation of fasting serum glucose followed by the blood samples collected at prefixed time intervals 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 h for detection of serum glucose levels and percentage reduction in serum glucose levels, which were calculated for all the groups. The animals were not killed; therefore, no euthanasia process was adopted. The animals were kept under washing period for 20 days to carry out the other experiment.

Induction of diabetes

Streptozotocin solution (45 mg/kg, interperitoneally) was prepared freshly at the time of administration in citrate buffer [9]. It was used within 10 min of its preparation. Rats of either sex weighing between 180 and 220 g were selected and fasted for 18 h before the experiment, and water was supplied ad libitum. The animals were kept in colony cages at ambient temperature of 28±2°C and relative humidity of 45–55% with a 12-h light/dark cycle. The streptozotocin solution has been prepared with 0.1 M citrate buffer solution, and the rats were intraperitoneally administered with 45 mg/kg of streptozotocin solution in a dark room.

Experimental study in diabetic rats

Overall, 13 groups of Wistar albino rats of either sex weighing 180–200 g were selected for the study. The animals were fasted for 18 h before commencing the experiment, with water provided ad libitum. The fasting was continued till completion of the experiment. For the determination of fasting blood glucose level, 0 h blood samples were collected.

Herb–drug interaction study in diabetic rats

In this study, diabetic rats of group 1 were administered with 0.1 M NaOH; groups 2, 3, and 4 were administered with 100, 200, and 400 mg/kg *A. vera*; groups 5, 6, and 7 were administered with ½ TD, 1 TD, and 2 TD glimepiride, respectively; group 8 was administered with *A. vera* 400 mg/kg+½ TD glimepiride; group 9 was administered with *A. vera* 400 mg/kg+1 TD glimepiride; group 10 was administered with *A. vera* 400 mg/kg+2 TD glimepiride to study the herb–drug interaction for one; similarly, group 11 was administered with *A. vera* 400 mg/kg+½ TD glimepiride; group 12 was administered with *A. vera* 400 mg/kg+1 TD glimepiride; and group 13 was administered with *A. vera* 400 mg/kg+2 TD glimepiride to observe the herb–drug interaction for the 8-day study, in which repeated dose of herb was administered for 8 days followed by a single dose of drug (8th day of the study).The blood samples were collected at prefixed

time intervals of 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 h for detection of serum glucose and insulin levels in the aforementioned studies. The percentage reduction in serum glucose levels at time t was calculated by using the following equation. The animals were not killed; therefore, no euthanasia process was adopted. The animals were kept under washing period for 20 days to carry out the other experiment.

$$\text{Percentage reduction in serum glucose at time } t = \frac{A-B}{A} \times 100.$$

where, A is serum glucose concentration at time 0 and B is serum glucose concentration at time t.

Chemiluminescence immunoassay method (insulin determination)

To carry out the assay of control and test in duplicate, all the microplate wells were formatted. Any unused microwell strips were replaced back into the aluminum bag, sealed, and stored at 2–8°C. Overall, 0.05 ml (50 µl) of the appropriate calibrators, controls, and samples was pipetted into the assigned wells. Then, 0.1 ml of the insulin tracer reagent was added to each well. For the proper mixing, microplate was swirled gently for 20–30 s. Plastic wrap was used to cover the plates, and they were incubated at room temperature for 60 min. The relative light units were read using a 96-well microplate laminator for 0.2–1.0 s per well. The results were read within 30 min of adding the stop solution. The microplate contents were discarded by aspiration or decantation. For decanting, tapping and blotting of the microplates with the use of dry adsorbent paper was done. Wash buffer (350 µl) was used to decant or aspirate. Manual or automatic plate washer can also be used. Working signal reagent (0.1 ml) was added to all the wells. In the same order the reagents were added to minimize reaction time difference between the wells. The wells were incubated in the dark for 5 min at room temperature [10].

Statistical analysis

All data obtained during the experimental period were subjected to the statistical analysis by using Graph Pad PRISM Software (GraphPad Software Inc., San Diego, California, USA) and Instat 3. Analysis of variance was used to find the significant differences groups followed by a post-hoc test, Tukey's multiple comparison test, or Dunnett's test.

Results

Acute oral toxicity study

The aqueous extract of *A. vera* was administered orally to different groups of mice at different dose levels. The animals were tested with 2000 mg/kg body weight, the extract has not showed any behavioral changes for 8 h and no mortality was observed for the period of 14 days.

Results of 1 day herb–drug interaction study

Influence of Aloe vera on the glimepiride in normal rats

The single dose administration of *A. vera* (400 mg/kg, postoperatively) and glimepiride ½ TD (0.036 mg/200 g, postoperatively), 1 TD (0.072 mg/200 g, postoperatively), and 2 TD (0.144 mg/200 g, postoperatively) alone significantly reduced serum glucose levels by 38.46, 34.22, 46.39, and 50.86%, respectively at 6 h. The maximum serum glucose reduction observed with glimepiride 2 TD after treatment of *A. vera* (400 mg/kg, postoperatively) produced 55.50% at 6 h after administration. All the combination groups shows significant reduction in serum glucose levels when compared with normal and 1 TD glimepiride. The Dunnett's test was applied to the paired data to find out the statically difference between combination (*A. vera*+glimepiride) group and glimepiride matching control group. The enhancement of glimepiride induced hypoglycemic effect by *A. vera* was statistically significant at all time intervals of the study, that is 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 h intervals. Since *A. vera* alters the serum glucose levels, it is likely to interfere with pharmacodynamics of glimepiride. When *A. vera* given in combination with glimepiride, it enhanced the hypoglycemic effect of glimepiride. The details of the results cited in Tables 1–3. The results were also observed in Figs 1–3. Based on this interaction exist in normal rats, the work is planned to be carry out in diabetic rats.

Influence of Aloe vera on the glimepiride in diabetic rats

As seen in normal rats, the diabetic rats also shown significant reduction in serum glucose levels. The single dose administration of *A. vera* (400 mg/kg, postoperatively) and glimepiride ½ TD (0.036 mg/200 g, postoperatively), 1 TD (0.072 mg/200 g, postoperatively), and 2 TD (0.144 mg/200 g, postoperatively) alone significantly reduced serum glucose levels 40.52, 34.22, 46.39, and 50.86% at 6 h. The maximum serum glucose reduction observed with glimepiride 2 TD after treatment of *A. vera* (400 mg/kg, postoperatively) produced 58.80% at 6 h. The combination of *A. vera* (400 mg/kg) with low dose of glimepiride (1/2 TD) produced reduction in serum glucose levels nearer to that observed with glimepiride 2 TD (0.144 mg/200 g, postoperatively) alone. The serum glucose reduction observed with glimepiride 2 TD after treatment of *A. vera* (400 mg/kg, postoperatively) produced 49.78% at 4 h similar to that observed with glimepiride 2 TD (0.144 mg/200 g, postoperatively)

Table 1 Hypoglycemic effect of *Aloe vera* in normal rats

Time (h)	Serum glucose (mg/dl) (mean±SEM)			% Reduction (mean±SEM)		
	Aloe vera (100 mg/kg)	*Aloe vera* (200 mg/kg)	*Aloe vera* (400 mg/kg)	*Aloe vera* (100 mg/kg)	*Aloe vera* (200 mg/kg)	*Aloe vera* (400 mg/kg)
0	89.15±1.02	88.62±1.02	88.78±0.84	–	–	–
1	86.09±0.50	85.12±1.00	84.77±0.91	3.39±0.80	3.95±0.48	4.51±0.60
2	81.69±0.56	80.30±0.79	75.96±1.38	8.30±1.35	9.35±0.91	14.37±2.05
3	77.86±0.55	76.99±0.91	68.89±1.42	12.61±1.05	13.09±1.03	22.34±1.98**
4	73.93±0.65	71.53±0.63	63.80±1.04	17.71±0.99**	19.23±1.19**	28.10±1.40**
6	70.10±0.64	67.26±0.78	54.57±1.73	20.63±1.45^{n}s	24.04±1.37^{n}s	38.46±2.28^{n}s
8	77.49±0.70	76.11±0.31	69.13±1.29	13.02±1.20**	14.05±1.11**	22.09±1.70**
10	79.81±0.45	79.04±0.60	74.02±1.53	10.42±0.93	10.79±1.09	16.57±1.99**
12	82.46±0.32	82.03±0.46	77.25±1.41	7.45±0.84	7.40±0.62	12.96±1.63
16	84.60±0.32	84.05±0.47	80.80±0.82	5.06±0.80	5.12±0.59	8.96±1.10
24	87.06±0.67	86.13±0.74	85.39±0.70	2.31±0.40	2.79±0.39	3.80±0.71

N=6. *Significant at *P* value less than 0.05. ***P* value less than 0.01. ****P* value less than 0.001 when compared with control group.

Table 2 Hypoglycemic effect of glimepiride in normal rats

Time (h)	Serum glucose (mg/dl) (mean±SEM)			% Reduction (mean±SEM)		
	½ TD GLIM	1 TD GLIM	2 TD GLIM	½ TD GLIM	1 TD GLIM	2 TD GLIM
0	87.77±1.15	88.65±0.96	88.95±2.23	–	–	–
1	84.01±0.93	83.71±1.03	82.61±2.09	4.26±0.31	5.58±0.33	7.12±0.25
2	77.55±1.19	74.81±0.57	73.71±1.98	11.63±0.95	15.59±0.51	17.16±0.34
3	72.08±1.45	67.09±0.71	65.80±1.34	17.84±1.65**	24.30±0.77**	25.98±0.41**
4	69.94±1.11	61.58±1.66	57.56±1.34	20.31±0.69**	30.51±0.89**	35.22±1.23**
6	63.78±1.01	51.47±0.53	45.57±1.36	27.29±1.24^{n}s	41.92±0.48***##	49.52±2.30***##ns
8	74.44±1.15	66.56±0.88	6264±2.02	15.18±0.72**	24.85±1.50**	29.54±1.67**
10	77.18±1.16	71.06±0.44	69.58±1.92	12.06±0.60	19.79±0.99**	21.75±1.28**
12	79.75±0.55	76.29±0.85	75.35±1.80	9.12±0.67	13.92±0.81	15.26±0.72
16	82.27±0.94	80.15±0.55	79.72±1.92	6.25±0.47	9.56±0.47	10.36±0.30
24	85.28±1.19	84.07±0.57	83.00±1.95	2.84±0.34	5.15±0.43	6.67±0.40

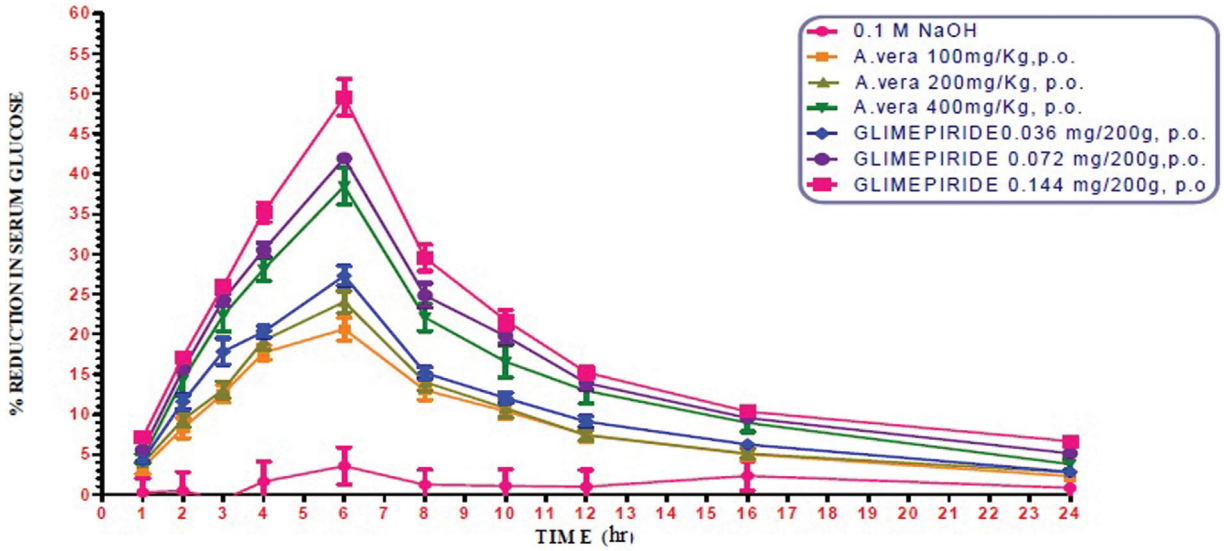
N=6. GLIM, glimepiride; TD, therapeutic dose. *Significant at *P* value less than 0.05. ***P* value less than 0.01. ****P* value less than 0.001 when compared to control group. ^{ns}P value more than 0.05. $^{\#}P$ value less than 0.05. $^{\#\#}P$ value less than 0.01. $^{\#\#\#}P$ value less than 0.001 when compared to glimepiride ½ TD, 1 TD, 2 TD group.

Table 3 Influence of single dose treatment of *Aloe vera* (400 mg/kg) on hypoglycemic activity of different dosage of glimepiride in normal rats

Time (h)	Serum glucose (mg/dl)			% Reduction		
	400 mg *Aloe vera*			400 mg *Aloe vera*		
	½ TD GLIM	1 TD GLIM	2 TD GLIM	½ TD GLIM	1 TD GLIM	2 TD GLIM
0	90.22±2.40	86.80±2.39	90.22±2.21	–	–	–
1	82.01±1.77	74.12±1.97	74.16±1.90	9.02±0.74	14.59±0.40	17.80±0.49
2	73.68±1.50	67.76±1.86	65.26±1.73	18.22±1.18	23.07±0.68	27.67±0.55
3	62.72±0.67	57.11±2.01	56.92±1.02	30.30±1.36**	34.26±0.81**	36.83±0.75**
4	59.04±0.54	49.96±1.49	47.51±1.82	34.34±1.71**	42.45±0.35**	47.42±0.88**
6	52.06±1.47	43.36±1.45	40.10±0.75	42.27±0.81***^{n}s	50.03±1.00***##$	55.50±0.57***##$$++
8	65.63±1.12	51.59±1.58	47.58±0.73	27.09±1.55**	40.22±0.66**	47.19±0.54**
10	68.24±1.26	57.29±1.51	54.80±1.38	24.21±1.49**	33.96±0.59**	39.25±0.43**
12	71.12±1.31	62.40±1.79	60.64±1.72	20.94±2.17	28.11±0.39	32.81±0.34
16	74.84±1.51	66.41±1.74	66.25±1.71	16.89±1.60	23.46±0.62	26.57±0.18
24	79.17±1.87	70.72±1.69	69.09±2.02	12.16±1.32	18.45±0.95	23.45±0.45

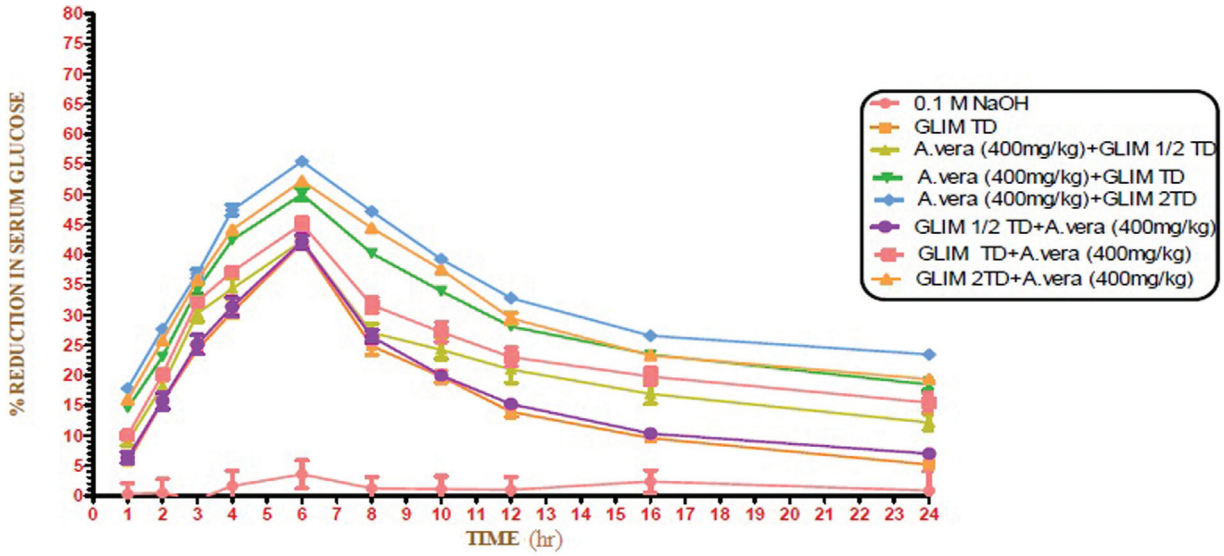
N=6. GLIM, glimepiride; TD, therapeutic dose. *Significant at *P* value less than 0.05. ***P* value less than 0.01. ****P* value less than 0.001 when compared to control group. ^{ns}P value more than 0.05. $^{\#}P$ value less than 0.05. $^{\#\#}P$ value less than 0.01. $^{\#\#\#}P$ value less than 0.001 when compared with glimepiride ½ TD, 1 TD, 2 TD group. $^{\$}P$ value less than 0.05. $^{\$\$}P$ value less than 0.01. $^{++}P<0.01$, when compared to 2 TD.

Figure 1



Percentage reduction in serum glucose with *Aloe vera* (100, 200, and 400 mg/kg) and glimepiride ½ TD, 1 TD, and 2 TD (0.036, 0.072, and 0.144 mg/200 g) in normal rats. TD, therapeutic dose.

Figure 2



Percentage reduction in serum glucose with single dose administration of *Aloe vera* (400 mg/kg) and glimepiride (0.036, 0.072, and 0.144 mg/200 g) in normal rats.

produced 50.86% at 6 h. The serum glucose reduction levels observed with glimepiride ½ TD (0.036 mg/200 g, postoperatively), 1 TD (0.072 mg/200 g, postoperatively), and 2 TD (0.144 mg/200 g, postoperatively) before and after treatment of *A. vera* (400 mg/kg, postoperatively) produce 44.45, 48.50, 54.27, and 47.6, 51.82, and 58.8%. The results were summarized in Tables 4–6. The results also explained with Figs 4–6.

Results of 8th day herb–drug interaction study

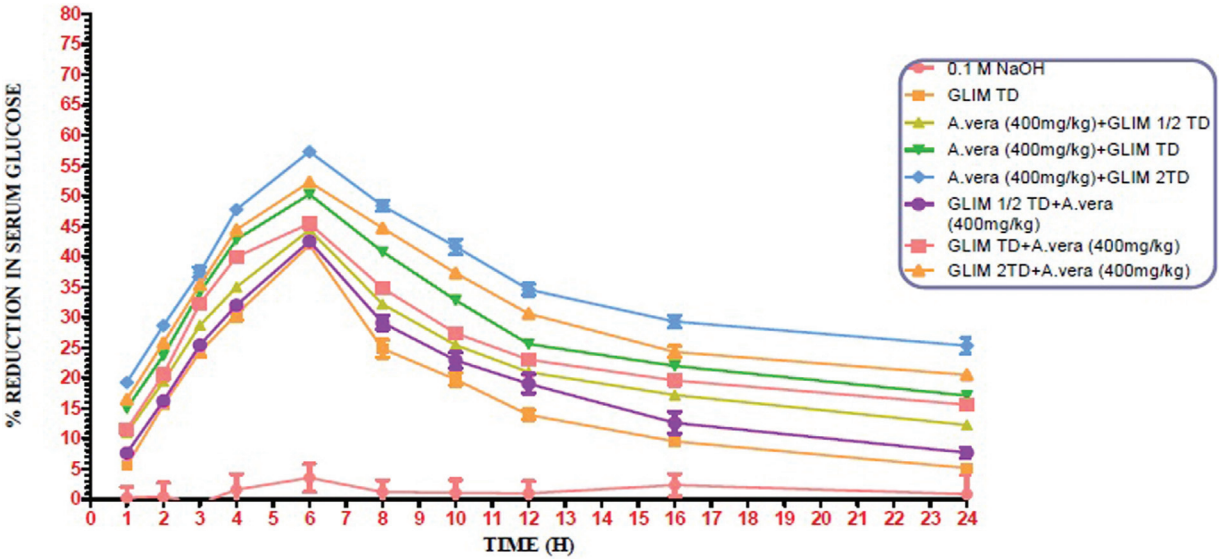
Influence of Aloe vera on the glimepiride in normal rats

When compared with vehicle treated control group, three different groups treated with single dose of *A. vera* (400 mg/kg, postoperatively) daily once for 8 days followed by single dose of glimepiride ½ TD, 1 TD, and 2 TD administered in respective groups 30 min later exhibited significant reduction in the serum glucose levels in normal rats. The details were summarized in Table 7.

Influence of Aloe vera on the glimepiride in diabetic rats

When compared with diabetic control group, three different groups treated with single dose of *A. vera* (400 mg/kg, postoperatively) daily once for 8 days followed by single dose of glimepiride ½ TD, 1 TD, and 2 TD administered in respective groups 30 min

Figure 3



Percentage reduction in serum glucose with repeated dose treatment repeated dose treatment of *Aloe vera*+single dose of glimepiride (½ TD, 1 TD, and 2 TD) in normal rats. TD, therapeutic dose.

Table 4 Influence of repeated dose treatment of *Aloe vera* (400 mg/kg) on hypoglycemic activity of single dosage of glimepiride (8th day)

Time (h)	Serum glucose (mg/dl)			% Reduction		
	400 mg *Aloe vera*			400 mg *Aloe vera*		
	½ TD GLIM	1 TD GLIM	2 TD GLIM	½ TD GLIM	1 TD GLIM	2 TD GLIM
0	77.21±3.21	80.46±2.27	78.76±2.20	–	–	–
1	68.74±2.85	68.36±1.87	63.59±1.95	11.01±0.20	15.02±0.23	19.28±0.57
2	62.24±2.54	61.37±1.70	56.13±1.34	19.42±0.27	23.72±0.09	28.67±0.63
3	55.07±2.26	52.96±1.40	49.23±1.06	28.69±0.47**	34.15±0.26**	37.41±0.88**
4	50.19±1.89	46.01±1.34	41.09±1.04	34.97±0.28**	42.82±0.19**	47.79±0.54**
6	42.86±1.51	49.95±0.89	33.58±0.98	44.44±0.32**##ns	50.29±0.63**##ns	57.32±0.72**##$$++
8	52.30±1.68	47.63±1.36	40.66±1.49	32.17±0.49**	40.80±0.25**	48.41±0.79**
10	57.53±2.07	54.03±1.49	45.98±1.76	25.43±0.57**	32.84±0.23**	41.65±1.19**
12	61.00±2.27	59.82±1.54	51.44±1.17	20.97±0.34	25.62±0.18	34.60±0.95
16	63.88±2.27	62.75±1.69	55.57±0.97	17.21±0.41	21.99±0.26	29.31±0.96
24	67.72±2.55	66.68±1.83	58.70±1.26	12.27±0.16	17.11±0.41	25.34±1.30

N=6. GLIM, glimepiride; TD, therapeutic dose. *Significant at *P* value less than 0.05. **P* value less than 0.01. ***P* value less than 0.001 when compared to control group. ^{ns}P value more than 0.05. $^{\#}P$ value less than 0.05. $^{\#\#}P$ value less than 0.01. $^{\#\#\#}P$ value less than 0.001 when compared to glimepiride ½ TD, 1 TD, and 2 TD group; ^{ns}P value more than 0.05. $^{\$}P$ value less than 0.05. $^{\$\$}P$ value less than 0.01. $^{\$\$\$}P$ value less than 0.001 when compared to glimepiride TD group.

later exhibited significant reduction in the serum glucose levels by Tukey's test are shown in the Table 8.

Effect of *Aloe vera* and glimepiride on serum insulin levels in diabetic rats by chemiluminescence method

When compared with diabetic control group (6.16 µu/ml) *A. vera* administered at dose of 400 mg/kg, postoperatively, glimepiride 1 TD (0.072 mg/200 g, postoperatively), and 2 TD (0.144 mg/200 g, postoperatively) is significant increase the serum insulin levels recorded as 9.01, 9.72, and 10.25 µu/ml except *A. vera* 100, 200 mg/kg and glimepiride ½ TD (0.036 mg/200 g, postoperatively) nearer to diabetic control group.

In 1 day interaction, all the combination groups shows significant increase in serum insulin levels when compared with diabetic control group. The maximum increase serum insulin levels observed with glimepiride 2 TD after treatment of *A. vera* (400 mg/kg, postoperatively) produced 14.10 µu/ml. In 8 days interaction study, all the combination groups shows significant increase in serum insulin levels when compared with diabetic control group. The maximum increase serum insulin levels observed with repeated treatment of *A. vera* (400 mg/kg, postoperatively) daily once for 8 days followed by administration with glimepiride 2 TD (0.144 mg/200 g, postoperatively) 30 min later

Table 5 Antidiabetic activity of *Aloe vera* in diabetic rats

Time (h)	Serum glucose (mg/dl)			% Reduction		
	Aloe vera (100 mg/kg)	*Aloe vera* (200 mg/kg)	*Aloe vera* (400 mg/kg)	*Aloe vera* (100 mg/kg)	*Aloe vera* (200 mg/kg)	*Aloe vera* (400 mg/kg)
0	313.2±7.09	300.7±6.04	302.0±7.47	–	–	–
1	294.0±7.26	279.6±6.02	277.3±7.24	6.13±0.51	6.99±0.85	8.17±0.39
2	275.6±5.04	264.6±5.70	253.6±6.41	11.90±1.42	11.96±0.85	16.01±0.45
3	261.3±4.65	248.7±6.28	227.7±7.36	16.45±1.38	17.27±1.18	24.66±0.64**
4	244.8±4.19	233.3±4.98	211.2±5.98	21.74±1.78**	23.39±0.19**	30.04±1.23**
6	220.2±4.85	204.5±5.85	179.7±5.97	29.63±1.22**^{n}s	32.00±1.04***#ns	40.52±1.04***#ns
8	235.1±7.31	220.2±6.35	204.3±5.25	24.93±1.47**	26.79±1.30**	32.29±1.06**
10	250.3±4.56	238.7±5.27	222.9±5.25	19.96±1.48	20.59±0.74**	26.16±0.64**
12	269.3±5.05	256.8±5.13	243.6±6.33	13.95±0.43	14.58±0.41	19.33±0.71
16	279.1±5.19	264.8±4.98	256.6±6.41	10.80±0.89	11.87±1.02	15.01±0.79
24	292.4±6.01	278.8±4.13	275.4±8.13	6.59±0.81	7.19±1.14	8.84±0.72

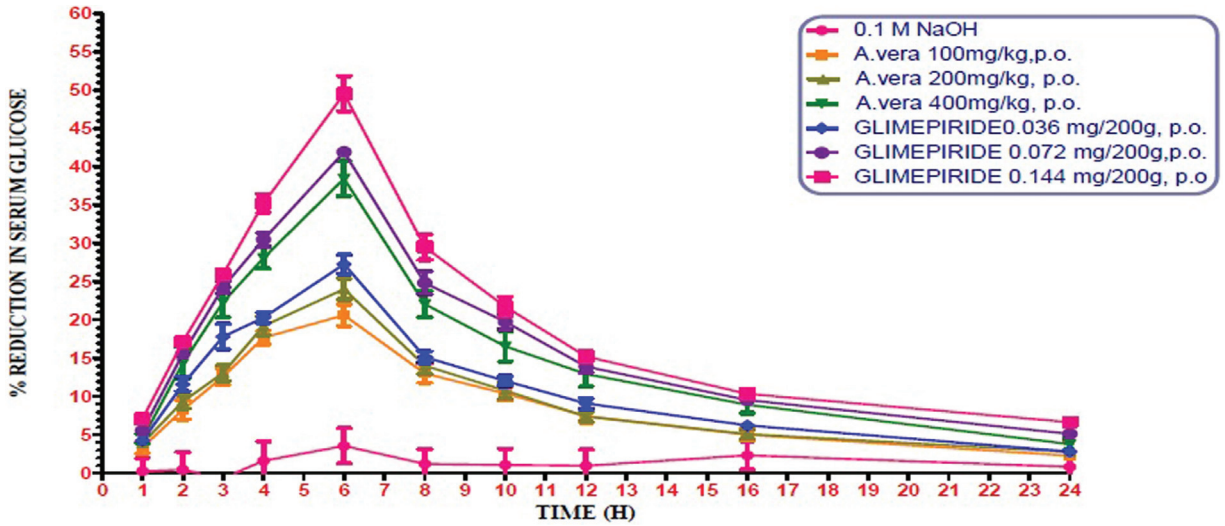
N=6. TD, therapeutic dose. *Significant at *P* value less than 0.05. **P value less than 0.01. ***P value less than 0.001 when compared to control group. ^{ns}P value more than 0.05. $^{\#}P$ value less than 0.05. $^{\#\#}P$ value less than 0.01. $^{\#\#\#}P$ value less than 0.001 when compared to glimepiride ½ TD, 1 TD, and 2 TD group; when compared to glimepiride TD group.

Table 6 Antidiabetic activity of glimepiride in diabetic rats

Time (h)	Serum glucose (mg/dl)			% Reduction		
	½ TD GLIM	1 TD GLIM	2 TD GLIM	½ TD GLIM	1 TD GLIM	2 TD GLIM
0	305.2±4.13	310.4±9.40	301.5±4.48	–	–	–
1	283.0±±4.17	283.7±7.62	274.8±4.10	7.27±0.66	8.55±0.71	8.86±0.51
2	260.2±3.79	256.6±5.08	245.0±5.49	14.72±0.46	17.19±0.96	18.77±0.65
3	233.8±3.79	226.4±4.81	216.2±4.80	23.36±0.97	26.95±0.97	28.29±0.84
4	218.3±2.42	197.5±5.11	185.8±4.17	28.44±0.59**	36.31±0.73**	38.4±0.63**
6	200.65±1.60	166.5±6.13	148.0±1.77	34.22±0.62**^{ns}	46.39±1.55***#ns	50.86±0.62***#ns
8	216.3±3.82	184.1±5.02	173.5±2.45	29.07±1.42**	40.51±1.80**	42.40±1.05**
10	229.1±2.29	201.2±6.92	192.7±2.67	24.87±0.99	35.02±2.26	36.07±0.43
12	249.9±3.34	236.4±6.82	219.4±2.99	18.08±0.63	23.75±1.58	27.19±0.53
16	263.2±3.12	253.5±6.44	236.9±2.65	13.74±0.61	18.22±1.32	21.38±1.02
24	281.8±3.34	270.8±6.29	257.1±4.35	7.59±1.42	12.56±2.10	14.74±0.47

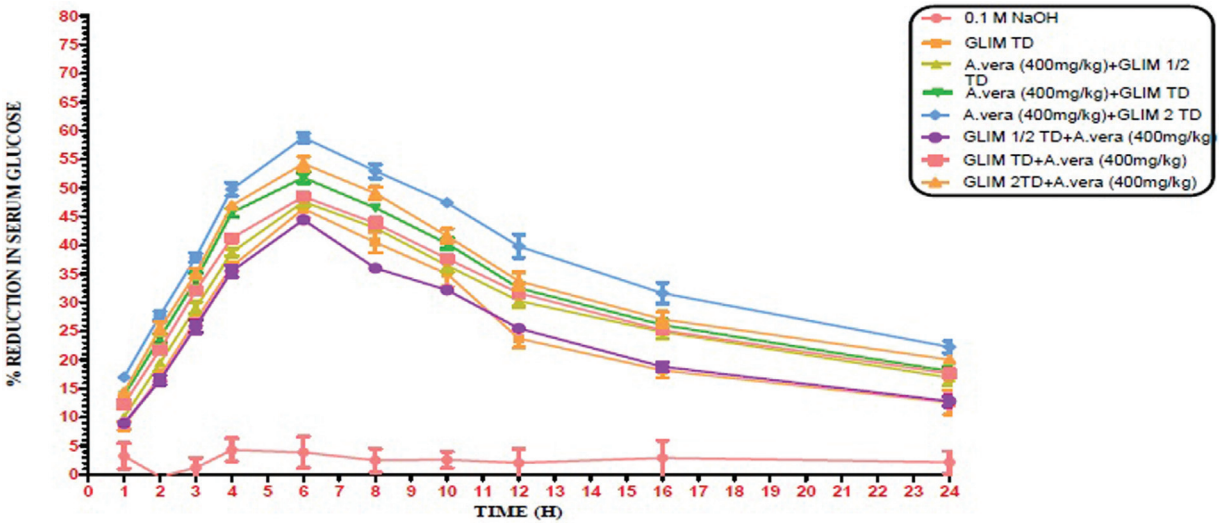
N=6. GLIM, glimepiride; TD, therapeutic dose. *Significant at *P* value less than 0.05. **P value less than 0.01. ***P value less than 0.001 when compared to control group. ^{ns}P value more than 0.05. $^{\#}P$ value less than 0.05. $^{\#\#}P$ value less than 0.01. $^{\#\#\#}P$ value less than 0.001 when compared to glimepiride ½ TD, 1 TD, and 2 TD group; when compared with glimepiride TD group.

Figure 4



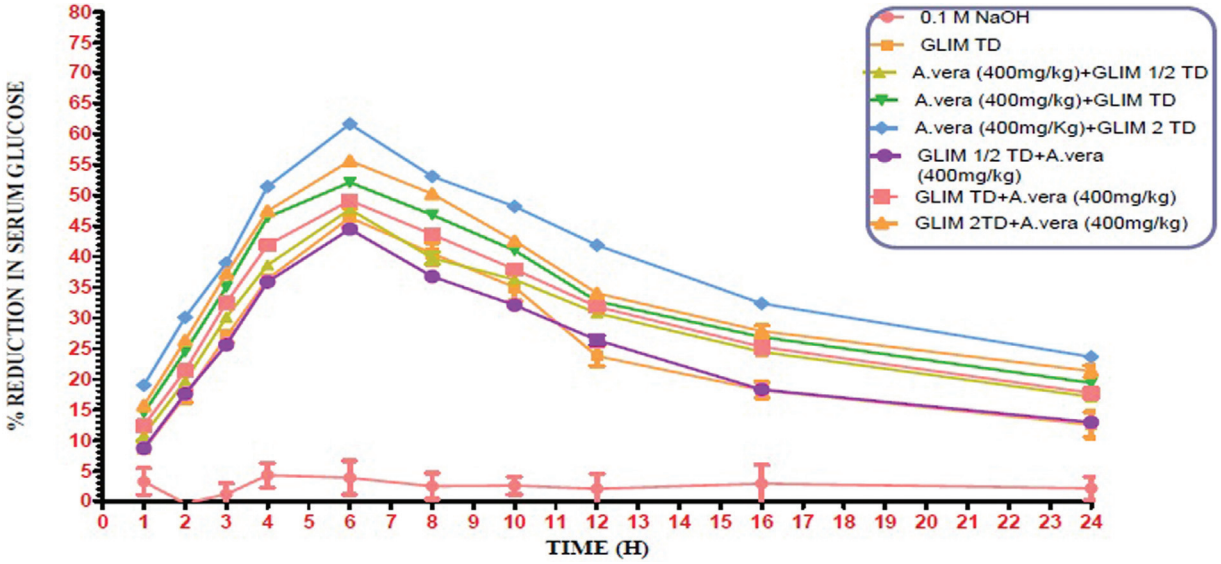
Percentage reduction in serum glucose with *Aloe vera* (100, 200, and 400 mg/kg) and glimepiride ½ TD, 1 TD, and 2 TD (0.036, 0.072, and 0.144 mg/200 g) in diabetic rats. TD, therapeutic dose.

Figure 5



Percentage reduction in serum glucose with single dose administration of *Aloe vera* (400 mg/kg) and glimepiride (0.036, 0.072, and 0.144 mg/200 g) in diabetic rats.

Figure 6



Percentage reduction in serum glucose with repeated dose treatment repeated dose treatment of *Aloe vera*+single dose of glimepiride (½ TD, 1 TD, and 2 TD) in diabetic rats. TD, therapeutic dose.

produced 22.25 µu/ml. as shown in Table 9, Figs 7 and 8.

Discussion

The present study was undertaken to evaluate possible herb–drug interaction if any between *A. vera* and glimepiride with single and repeated dose treatment of *A. vera* on hypoglycemic and antidiabetic activity of glimepiride in normal and diabetic rats. The results indicate that animals administered with single dose of *A. vera* (400 mg/kg, postoperatively) single dose treatment of glimepiride (½ TD, 1 TD, and 2 TD) in normal and diabetic rats produced a significant hypoglycemic and antidiabetic activity. The maximum serum glucose reduction observed with glimepiride 2 TD after treatment of *A. vera* (400 mg/kg, postoperatively) at 6 h of the study. The combination of *A. vera* (400 mg/kg) with low dose of glimepiride (1/2 TD) produced glucose reduction nearer to that observed with glimepiride 2 TD (0.144 mg/200 g, postoperatively) dose. The results observed that *A. vera* when administered prior to glimepiride enhanced the hypoglycemic activity of the later. The enhancement of glimepiride induced hypoglycemic effect by *A. vera* was statistically significant at all time intervals of the study. The

Table 7 Influence of single dose treatment of *Aloe vera* (400 mg/kg) on antidiabetic activity of glimepiride in diabetic rats

Time (h)	Serum glucose (mg/dl)			% Reduction		
	400 mg *Aloe vera*			400 mg *Aloe vera*		
	½ TD GLIM	1 TD GLIM	2 TD GLIM	½ TD GLIM	1 TD GLIM	2 TD GLIM
0	301.7±7.65	295.4±7.00	297.4±5.93	–	–	–
1	271.4±7.08	254.4±5.18	246.8±5.08	10.33±0.44	13.82±0.46	17.01±0.39
2	242.9±6.63	225.0±6.69	214.8±4.68	19.49±0.40	23.87±0.71	27.76±0.73
3	214.1±7.05	194.9±3.60	185.0±5.76	29.07±1.00**	33.91±1.26**	37.86±0.77**
4	184.5±4.17	160.5±5.11	149.61±5.81	38.78±0.69**	45.70±0.73**	49.78±1.13**
6	158.2±5.62	142.3±4.39	122,6±4.12	47.6±0.89***##	51.82±0.91***##ns	58.8±0.83***##$$++
8	171.7±3.68	157.8±3.56	140.1±5.45	43.04±0.45**	46.84±0.44**	52.95±1.26**
10	191.2±1.85	176.1±3.87	156.5±7.70	30.48±1.18**$$	40.31±0.91**	47.45±0.02**
12	209.9±4.08	199.0±3.18	179.2±8.37	30.31±1.02	32.53±0.82	39.84±2.03
16	228.4±4.78	218.2±6.35	203.4±7.38	24.91±1.18	26.15±0.91	31.66±1.80
24	250.1±3.96	242.1±6.50	231.1±5.51	16.94±1.35	18.05±0.75	22.27±1.03

N=6. GLIM, glimepiride; TD, therapeutic dose. *Significant at *P* value less than 0.05. **P value less than 0.01. ***P value less than 0.001 when compared to control group. ^{ns}P value more than 0.05. $^{\#}P$ value less than 0.05. $^{\#\#}P$ value less than 0.01. $^{\#\#\#}P$ value less than 0.001 when compared to glimepiride ½ TD, 1 TD, and 2 TD group; when compared with glimepiride TD group. $^{\$\$}P$ value less than 0.01. $^{+}$ $^{+}P<0.01$, when compared to 2 TD.

Table 8 Influence of repeated dose treatment of *Aloe vera* (400 mg/kg) on antidiabetic activity of glimepiride in diabetic rats (8th day)

Time (h)	Serum glucose (mg/dl)			% Reduction		
	400 mg *Aloe vera*			400 mg *Aloe vera*		
	½ TD GLIM	1 TD GLIM	2 TD GLIM	½ TD GLIM	1 TD GLIM	2 TD GLIM
0	209.0±8.97	194.7±6.04	195.7±8.23	–	–	–
1	186.2±8.16	166.5±5.37	158.3±5.80	10.88±0.94	14.49±0.60	19.02±0.47
2	167.9±7.25	147.0±4.87	136.7±5.89	19.67±0.60	24.50±0.60	30.11±0.49
3	145.9±6.00	126.5±4.04	119.5±5.56	30.12±0.60**	35.04±0.32**	38.99±0.30**
4	128.4±5.98	104.2±2.75	94.92±3.82	38.61±0.54**	46.44±0.29**	51.45±0.66**
6	109.3±4.94	93.14±2.54	75.17±4.12	47.73±0.39***##ns	52.14±0.47***##ns	61.69±0.51***##$$++
8	125.8±5.33	103.4±2.31	91.91±4.91	39.77±0.91**	46.81±0.74**	53.13±0.60**
10	133.1±4.92	114.8±3.22	101.4±4.67	36.22±0.66**	41.02±0.37**	48.02±0.27**
12	144.7±6.60	130.9±3.42	113.8±5.25	30.79±0.53**	32.79±0.44	41.87±0.68
16	157.9±6.74	142.3±4.00	132.2±4.98	24.45±0.59	26.88±0.41	32.36±0.70
24	173.1±7.15	157.0±4.97	149.4±6.29	17.11±0.60	19.38±0.45	23.63±0.22

N=6. GLIM, glimepiride; TD, therapeutic dose. *Significant at *P* value less than 0.05. **P value less than 0.01. ***P value less than 0.001 when compared to control group. ^{ns}P value more than 0.05. $^{\#}P$ value less than 0.05. $^{\#\#}P$ value less than 0.01. $^{\#\#\#}P$ value less than 0.001 when compared to glimepiride ½ TD, 1 TD, and 2 TD group; when compared to glimepiride TD group. $^{\$\$}P$ value less than 0.01. $^{++}P<0.01$, when compared to 2 TD.

Table 9 Serum insulin levels on Single and repeated dose administration of *Aloe vera* and glimepiride in diabetic rats (chemiluminescent immune assay method)

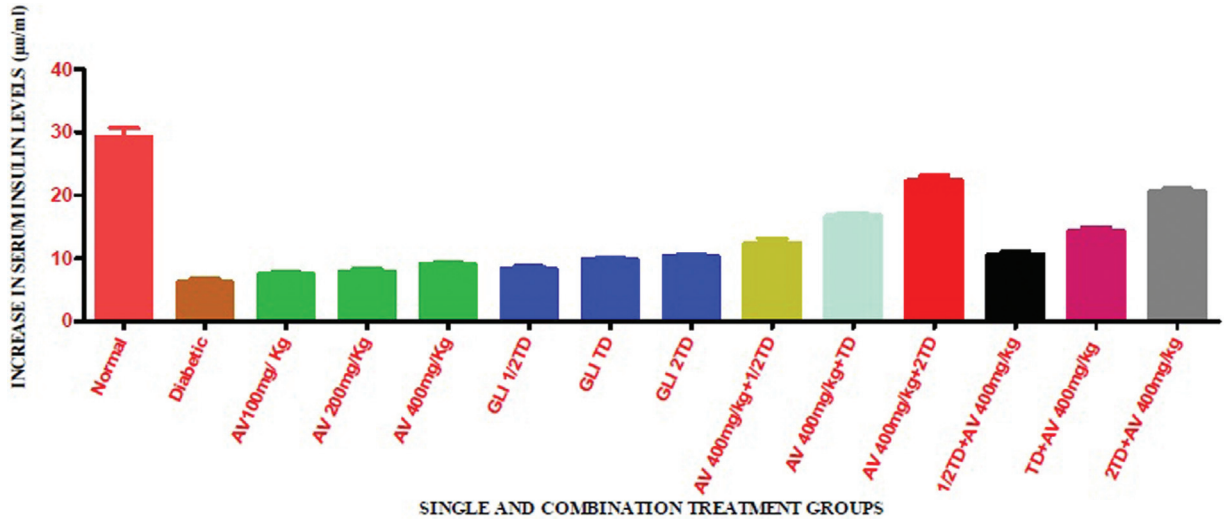
Groups	1st day (mean ±SEM)	8th day (mean ±SEM)
Aloe vera 400 mg/kg+½ TD GLIM	9.72±0.21**	12.39±0.74**
Aloe vera 400 mg/kg+1 TD GLIM	11.97±0.58**	16.69±0.4**
Aloe vera 400 mg/kg+2 TD GLIM	14.10±0.56**	22.25±0.83**

N=6. GLIM, glimepiride; TD, therapeutic dose. *Significant at, *P* value less than 0.01. **P value less than 0.01.

results show that the animals administered with *A. vera* (400 mg/kg) daily once for 8 days and ½ TD, 1 TD, and 2 TD of glimepiride were administered respectively after 30 min later in normal and diabetic rats and similarly in a reverse study, produced a significant hypoglycemic and antidiabetic activity. When compared with diabetic control group, all treated with a combination of *A. vera* and glimepiride groups shown a significant reduction in the serum glucose levels.

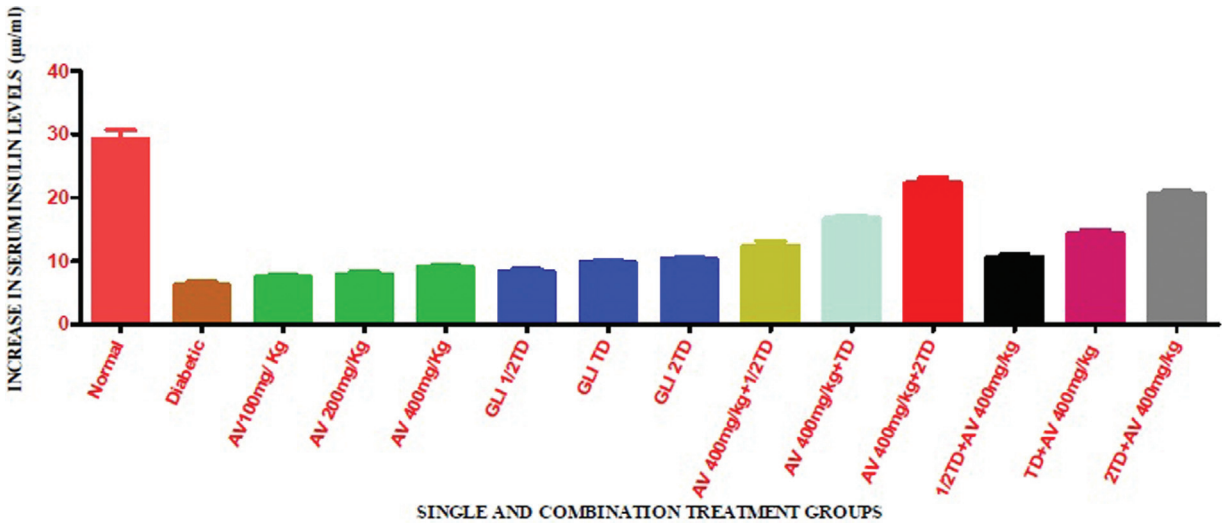
In the present study results revealed that a significant increase serum insulin levels was observed in diabetic rats when treated with combination of *A. vera* and glimepiride when compared with single drug/herb treated groups. It is possible that *A. vera* may initiate cell proliferation, since it has been reported that pancreatic endocrine cells have the potential to proliferate after induction of diabetes with streptozotocin [11]. It was reported that glimepiride has effect on the β-cells and increase the insulin

Figure 7



Serum insulin levels in diabetic rats for 1 day study.

Figure 8



Serum insulin levels in diabetic rats for 8 days study.

secretion [12]. Therefore, the combination of *A. vera* (400 mg/kg) with glimepiride 2 TD (0.144 mg/200 g, postoperatively) caused significant increase in serum insulin levels than individual treatment with glimepiride confirmed that extra increase in insulin levels are due to *A. vera* only. The results are on par with the statement mentioned above for *A. vera* for its effect on K^+ channels. The combination of *A. vera* and glimepiride could have resulted in increased hypoglycemic effect in the group treatment of these two combinations in streptozotocin-induced diabetes when compared with the effect observed with individual treatment. The results of the present study indicate that combining *A. vera* and glimepiride could lead to reduction in the dose of glimepiride, which may minimize the adverse effects as well as maintain enhanced therapeutic hypoglycemic effect. At the same time, proper precaution and care should be exercised to avoid severe hypoglycemia that may occur due to combination of these agents since both cause release of insulin.

Conclusion

The present study concluded that, the aqueous extract of *A. vera* augmented the hypoglycemic action of glimepiride. The groups treated with combination of these two showed a significant better effect compared with individual herb/drug treatments. Further, the combination of *A. vera* with high dose of glimepiride showed maximum hypoglycemia

when compared to selected doses of herb/drug treatments. This confirms an herb–drug interaction between *A. vera* and glimepiride.

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Authors' contributions: Dr Prasenjit Mondal, Kola Venu, and Shaik Chanbasha designed the entire project work and carried acute toxicity study and herb–drug, interaction study in normal rats. Dr Manish Kumar Thimmaraju and Kola Venu induced the diabetes to the animal and carried out herb–drug, interaction study in diabetic rats 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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Nil.

Conflicts of interest

There are no conflicts of interest.

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