ISSN 2475-5451

Research Article

International Journal of Diabetes & Metabolic Disorders

Hypoglycemic Activity of Ethanol Extract of Jasminum Grandiflorum Flowers in Vivo and Cytotoxicity of Its Chloroform Isolate in Vitro

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Submitted: 06 Apr 2018; **Accepted**: 13 Apr 2018; **Published**: 28 May 2018

Abstract

Ethno pharmacological relevance: *Traditionally different parts of Jasminum grandiflorum have been used to treat various ailments, including diabetes. However, antidiabetic potential of Jasminum grandiflorum on animal models of diabetes have not been evaluated.*

Aim of the study: *The objective of this study was to determine antidiabetic potential of ethanol extract of leaves and flowers of Jasminum grandiflorum, and different fractions of the flower extract in rodent model of streptozotocin-induced diabetes.*

Materials and methods: *Ethanol extract of both leaves and flowers of Jasminum grandiflorum were screened for the presence of various phytochemicals followed by acute and sub-acute toxicity in rats. Effect of Jasminum grandiflorum leaf and flower extracts on blood glucose level in normal albino rats, in glucose-overloaded healthy albino rats, and in streptozotocin-induced diabetic rats was evaluated. Furthermore, based on preliminary results, fractionalization of the flower extract was carried out using petroleum ether, ethyl acetate, methanol, and chloroform. Different fractions were further tested for hypoglycemic activity in streptozotocin-induced diabetic rats.*

Results: *Preliminary phytochemical evaluation suggested presence of various antidiabetic metabolites in both the extracts and were found to safe up to 5000 mg/kg dose. Flower extract (500 mg/kg, p.o.) demonstrated significant hypoglycemic effect than leaf extract (500 mg/kg, p.o.) in normal rats, glucose-overloaded rats, and streptozotocin-induced diabetic rats when compared to control. Long-term effect of different fractions of ethanol extract of Jasminum grandiflorum flowers in streptozotocin model suggested that all four fractions were able to reduce blood glucose level in a time-dependent manner at 200 mg/kg dose with chloroform fraction being highly significant ($p<0.001$) amongst all when compared to diabetic untreated rats. Chloroform isolate from Jasminum grandiflorum flowers demonstrated enhanced glucose uptake and dose-dependent cytotoxicity in L6 cell line.*

Conclusion: *The ethanol extract of Jasminum grandiflorum flowers as well as its various fractions have potential therapeutic value in treating diabetes, which may be due to the presence of various antidiabetic metabolites, by enhancing insulin secretion and antioxidant defense. These observations rationalize its use as ethnomedicine and hence can be considered in treating diabetes.*

Keywords: Antidiabetic Activity, Jasminum Grandiflorum, Ethanol Extract, Streptozotocin, Chloroform Isolate, Cytotoxicity, L6 Cell Line, Glucose Uptake

Introduction

According to the World Health Organization (WHO), diabetes mellitus or simply termed as 'diabetes' is a metabolic disorder of multiple etiology, characterized by chronic hyperglycemia with altered metabolism of carbohydrates, fats, and proteins-resulting from insufficient action of insulin, which may be due to impaired insulin secretion or increased insulin resistance, or both [1, 2]. Diabetes is no more epidemic but has turned into pandemic, which

is estimated to be affecting ~10% of the world population, as per the worldwide survey. The prevalence of diabetes is expected to increase by 69% by 2030 and the occurrence is projected to be high in countries like India, China, and the USA [3, 4].

Globally, both men and women are affected by diabetes similarly; however, it is slightly higher in men of < 60 years of age, while women are affected at older ages. Overall, diabetes prevalence is higher in men, but there are more women with diabetes than men [4, 5]. In case of Indian scenario, the country has more diabetics than any other nation in the world, according to the International Diabetes Foundation [6, 7]. Currently, more than 50 million Indians are affected by diabetes and nearly one million patients are dying every year because of diabetes and related complications. By 2030, number of people diagnosed with diabetes is expected to be 79.4 million compared to 40.6 million in 2006 [5].

Patients with all forms of diabetes of sufficient duration, including insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM), are vulnerable to several complications ranging from diabetic ketoacidosis, hyperosmolar nonketonic coma, and hypoglycemia (acute metabolic complications) to atherosclerosis, diabetic microangiopathy, diabetic nephropathy, diabetic retinopathy, and infections (late systemic complications). In addition, diabetes is also accompanied by a substantial increase in atherosclerotic deposition in large vessels, including cardiac, cerebral, and peripheral vascular disease (i.e. cardiovascular diseases) [8].

The pernicious effects of diabetes have been found to be mediated through oxidative stress, which is associated with increased production of reactive oxygen species (ROS) and impaired antioxidant defense mechanism, thus leading to lipid peroxidation, alteration in the status of antioxidant enzymes, decreased ascorbic acid levels, and impaired glutathione metabolism. Increased production and ineffective scavenging of ROS is thought to play a critical role in diabetes [9-11]. Alternatively, elevated blood glucose level would lead to increased formation and accumulation of advanced glycation end products (AGEs) and sorbitol concentration, which play an important role in diabetic complications, such as retinopathy, neuropathy, and renal dysfunction [12].

The use of plant by man for the treatment of diseases is an age-long practice. Diabetes mellitus was known in ancient times and some medicinal plants have been extensively used for its control in traditional systems of medicine, such as Ayurveda [3]. The oral antihyperglycemic agents currently used in the clinical practice have characteristic profiles of serious side effects. This has led to increasing demand for herbal products with antidiabetic activity [13, 14]. The WHO has recommended the evaluation of traditional medicinal plants for treating diabetes as they are safe and effective with less or no side effects, and are considered to be excellent candidates for oral therapy [15].

Jasminum grandiflorum of family Oleaceae, also known variously as the Spanish jasmine, Royal jasmine, Catalonian jasmine, among others, is a species of jasmine native to South Asia. In India, it is widely used as an Ayurveda herbal medicine. It is closely related to, and sometimes treated as merely a form of Jasminum officinal [16-18]. Traditionally, different parts of Jasminum grandiflorum have been used in Ayurveda for the treatment of various ailments, including chronic constipation, flatulence, dysmenorrhea, amenorrhea, ringworm, skin diseases, ulcers, giddiness, and diabetes [19, 20]. Several investigators have evaluated pharmacological activities of different parts of the plant, including anti-ulcer, antibacterial, antiviral, wound healing, antihypertensive, and anticancer and antioxidant [18, 21-27]. However, perusal of literature survey revealed that antidiabetic potential of Jasminum grandifluram has not been studied in vivo as yet. Therefore, the current research work was carried out to evaluate ethanol extracts of leaf and flower of Jasminum grandiflorum, various solvent fractions for hypoglycemic activity in vivo, while chloroform isolate from ethanol extract of flowers of Jasminum grandiflorum was assessed for antidiabetic activity in vitro.

Materials and Methods

Plant material

The leaves and flowers of Jasminum grandiflorum were collected from the surrounding gardens of Hiriyur (latitude: 13.9438° north and longitude: 76.6161° east), Karnataka, India, and were identified and authenticated by Prof. P. Channabasappa, Botanist, Sree Siddaganga Science College, Tumkur, Karnataka, India. A herbarium specimen (Voucher No. MCP/HS/17) was preserved in the college museum for future reference.

Preparation of the extracts

The leaves and flower were shade-dried separately at room temperature for 15 days and pulverized. The sieved powder was further subjected to hot continuous Soxhlet extraction with 70% ethanol for 24 h cycle at 40-50 °C. Excessive solvent was removed by solvent distillation apparatus and residue was concentrated by using Lyotrap dryer.

Preliminary phytochemical screening

Qualitative chemical tests were conducted in order to identify various phytoconstituents.Phytochemical examinations were carried out for both leaf and flower extracts separately for the presence of glycosides, alkaloids, flavanoids, tannins, resins, terpenoids, saponins, and phenolic compounds as per the standard methods [28].

Animals

For pharmacological experiments, male albino rats (180-220 g) bred at Bioneeds, Tumkur, India, were used. Animals were acclimatized and were maintained under standard condition (air-conditioned rooms with optimal air changes per hour, relative humidity, temperature, and elimination cycle set to 12 h light and 12 h in dark) in an animal house approved by the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), Mallige College of Pharmacy, Bengaluru, India (Reg. No. 1432/PO/a/11/CPCSEA/047/2015-16).

Dosage of the extracts and drug solutions

The ethanol extracts of leaf and flower of Jasminum grandiflorum were suspended in 1% Tween-80 solution. All the preparations (doses) were freshly prepared on the day of experimentation and administered to the animals. Suspension of glibenclamide (standard drug) was prepared by using Tween-80 (1%) solution.

Toxicity tests

The oral acute and sub-acute toxicity studies were determined in 40 healthy Wistar albino rats of both sexes as per OECD guidelines

425 [29]. Briefly, animals randomly divided into four groups and were fasted overnight before the study. Group I was treated with ethanol extract of Jasminum grandiflorum leaves (5000 mg/kg, p.o.), while group II received normal saline. Similarly, group III and IV received ethanol extract of Jasminum grandiflorum flowers (5000 mg/kg, p.o.) and normal saline, respectively.

Screening for antidiabetic activity

Effect of Jasminum grandiflorum leaf and flower extracts on blood glucose level in normal albino rats

In this experiment influence of Jasminum grandiflorum leaf and flower extracts on blood glucose level in healthy albino rats was evaluated [30]. Twenty-four male albino rats were divided into four groups containing six animals each and marked conveniently. The animals were fasted for 18 h before commencing the experiment. During this period, rats were provided with watered libitum. The fasting was continued till the completion of the experiment. Animals were administered with the vehicle (group-I), glibenclamide, standard drug (5 mg/kg, p.o.; group-II), leaf extract or flower extract of Jasminum grandiflorum (500 mg/kg, p.o.; group-III and group-IV, respectively). Blood samples were collected from tail tip at 0, 0.5, 1, 2, 4, and 6 h after the treatment. The blood samples were analyzed for blood glucose level by using glucometer. The percentage reduction in blood glucose level was calculated.

Effect of Jasminum grandiflorum leaf and flower extracts on blood glucose level in glucose-overloaded healthy albino rats

In this experiment oral glucose tolerance test (OGTT) was studied in glucose-overloaded male albino rats [31]. Animals were divided into four groups containing six animals each and marked conveniently. The animals were fasted overnight before the commencement of the experiment. During this period, rats were provided with water ad libitum. The rats of group I were treated with vehicle, group II received glibenclamide (5 mg/kg, p.o.), and animals from group III and IV were treated orally with 500 mg/kg of leaf and flower extracts, respectively. After 30 min. of drug treatment, all the animals were fed with glucose (4 g/kg) and blood glucose level was determined after 0.5, 1, 2, and 4 h of the glucose load. Blood glucose level was estimated by using a commercial glucometer and the percentage reduction in blood glucose was calculated.

Evaluation of antidiabetic potential of leaf and flower extracts of Jasminum grandiflorum in streptozotocin-induced diabetic rats Induction of diabetes

Male albino rats were selected and fasted for 18 h with water ad libitum. The rats were administrated with streptozotocin (80 mg/kg, i.p.) dissolved in normal saline (0.9% w/v NaCl in distilled water). The rats were treated with 20% glucose solution intraperitoneally after 6 h and were kept on 5% glucose solution bottles in their home cages for the next 24 h to prevent hypoglycemia. The blood samples were collected after 24 h by slicing tip of the tail and blood glucose levels were determined by using a commercial digital glucometer. Rats with blood sugar level more than 300 mg/dl were considered to be diabetic. Animals were allowed to stabilize for 4 days and further employed in the study [32].

Treatment protocol

Thirty male diabetic albino rats were selected and divided into five groups containing six animals each and marked conveniently. The animals were fasted for 18 h before beginning of the experiment. Animals were provided with water ad libitum. The fasting was continued till the completion of the experiment. Group I served as normal control (treated with normal saline), group II was diabetic control (received only vehicle), group III received glibenclamide (5 mg/kg, p.o.), group IV received leaf extract of Jasminum grandiflorum (500 mg/kg, p.o.), and group V received flower extract of Jasminum grandiflorum (500 mg/kg, p.o.).

The treatment was given following oral administration by gastric intubation, using a force-feeding needle. Blood glucose was estimated by withdrawing blood samples from tail tip onday 0, 7, 14, 21, and 28 of the treatment. The results obtained were recorded and percentage reduction in blood glucose was calculated.

Procedures for histopathology

At the end of the experiment (i.e. on day 28) animals were sacrificed by cervical dislocation and pancreas were immediately collected for histopathological investigation. With the help of a magnifying glass gross appearance and color change in the internal organs were examined for the sacrificed animals. The tissues were instantly fixed in 10% phosphate-buffered formalin and embedded in paraffin blocks and sectioned into 5 μm thin sections using microtome. Sections were stained with hematoxylin and eosin and examined under a light microscope (Nikon, Tokyo, Japan) [32].

Fractionalization of flower extract of Jasminum grandiflorum

Preliminary study results suggested that leaf extract did not show significant antidiabetic activity when compared to glibenclamide-treated group. However, flower extract showed potent hypoglycemic activity equivalent to the standard drug. Thus, flower extract of Jasminum grandiflorum was further considered for fractionalization.

Preparation of different fractions of flower extract of Jasminum grandiflorum

Four fractions of flower extract of Jasminum grandiflorum were prepared using solvents like petroleum ether, ethyl acetate, methanol, and chloroform upon verification of phytoconstituents present in the extract. Dried powders of the flower extract were extracted by sox let extraction method using ethanol. Residue obtained was further fractionated using petroleum ether, ethyl acetate, methanol and chloroform. All these fractions were evaporated to obtain concentrated fraction.

Evaluation of antidiabetic activity of different fractions of flower extract of Jasminum randiflorum

Induction of diabetes was done as described previously [32]. Experimental procedure involved randomization of 36 male diabetic albino rats into six groups containing six animals each and were marked conveniently. The animals were fasted for 18 h before beginning of the experiment. Animals were provided with water ad libitum. The fasting was continued till the completion of the experiment. Group I was diabetic control (received only vehicle), group II received glibenclamide (5 mg/kg, p.o.), group III received petroleum ether fraction (200 mg/kg, p.o.), group IV received ethyl acetate fraction (200 mg/kg, p.o.), and group V received methanol fraction (200 mg/kg, p.o.) and group VI received chloroform fraction (200 mg/kg, p.o.).

The treatment was given following oral administration by gastric intubation, using a force-feeding needle. Blood glucose was estimated by withdrawing blood samples from tail tip on day 0, 7, 14, 21, and 28 of the treatment. The results obtained were recorded

and percentage reduction in blood glucose was calculated.

Isolation of antidiabetic constituent from ethanol extracts of Jasminum grandiflorum flowers

Further to evaluation of antidiabetic activity of different fractions the identified active fraction was separated by means of thin layer chromatography (TLC) using silica gel as stationary phase and mixture of solvents chloroform: acetone, chloroform: ethyl acetate, and chloroform: acetone in the ratio of 4:1, 3.5:1.5, and 4.5:0.5, respectively as the mobile phase. Pre-coated TLC plates were used and the fraction bands were identified under UV lamp and by spraying with concentrated sulphuric acid-vanillin reagent. The isolated bands (RF 0.48, 0.69, and 0.51) were scrapped off from the TLC plates, dissolved in absolute methanol, filtered and concentrated to dryness.

In vitro evaluation of cytotoxicity and glucose uptake activity of chloroform isolate

Cell line and culture medium

Cell line L6 (Rat muscle) was cultured in DMEM-HG media supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in a humidified atmosphere of 5% CO2 at 37 °C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates.

Determination of cell viability by MTT Assay

The monolayer cell culture was trypsin zed and the cell count was adjusted to 1.0x105 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of chloroform isolate obtained from ethanol extract of Jasminum grandiflorum flowers (1.56-50 µg/ml) were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37 °C for 3 days in 5% CO2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the test solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37o C in 5% CO2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed Formosan. The absorbance was measured using a micro plate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line [33].

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} X100$$

Glucose uptake assay

Glucose uptake activity of the test substance was determined in differentiated L6 cell line. In brief, the 24 h cell cultures with 70-80% confluences in 40mm petriplates were allowed to differentiate by maintaining in DMEM with 2% FBS for 4-6 days. The extent of differentiation was established by observing multinucleation of cells. The differentiated cells were serum starved overnight and at the time of experiment cells were washed with HEPES buffered Krebs Ringer Phosphate solution (KRP buffer) once and incubated with KRP buffer with 0.1% BSA for 30min. at 37 °C. Cells were treated with different non-toxic concentrations of test and standard drugs for 30 min. along with negative controls at 37 °C. D-glucose solution was added simultaneously to each well and incubated at 37 °C for 30 min. After incubation, the uptake of the glucose was terminated by aspiration of solutions from wells and washing thrice with ice-cold KRP buffer solution. Cells were lysed with 0.1M NaOH solution and an aliquot of cell lysates were used to measure the cell-associated glucose. The glucose levels in cell lysates were measured using glucose assay kit (ERBA). Two independent experimental values in duplicates were taken to determine the percentage enhancement of glucose uptake over controls [34, 35].

Statistical analysis

All the experimental results were expressed as mean ± SEM and assessed for statistical significance using one-way analysis of variance (ANOVA) followed by Turkey multiple comparison tests.

Results

Preliminary phytochemical screening

Phytochemical analysis of ethanol extract of Jasminum grandiflorum leaf and flower confirmed the presence of different chemical constituent, viz. glycosides, alkaloids, flavanoids, tannins, resins, terpenoids, saponins, and phenolic compounds.

Toxicity tests

Both leaf and flower extracts of Jasminum grandiflorum were found to be safe with no reported sign and symptoms when animals were closely monitored immediately and then after 0.5, 1, 2, 4, 6 h, and thereafter daily up to 14 days.

Effect of Jasminum grandiflorum leaf and flower extracts on blood glucose level in normal albino rats

(Table 1) summarizes blood glucose-lowering effect of Jasminum grandiflorum leaf and flower extracts in normal albino rats. The leaf extract showed hypoglycemic activity only at 2 h and 4 h, while flower extract demonstrated a significant reduction in blood glucose levels from first hour through 6 h in comparison to normal control. The percentage reduction in the blood sugar level was found to be better in flower extract-treated group (25.67%) than leaf extract (18.63%) after 6 h of the treatment. The standard drug glibenclamide reduced the blood glucose level significantly in normal albino rats at all-time points and showed 57.86% reduction in blood glucose level at the end of the experiment.

Table 1: Effect of Jasmine grandiflorum leaf and flower extracts on blood glucose level in normal albino rats.

Treatment	Fasting blood glucose (mg/dL)						Reduction in blood glucose(%)					
	0.0	0.5	1.0	2.0	4.0	6.0	0.0	0.5	1.0	2.0	4.0	6.0
	Time (h)						Time (h)					
Normal control	78.17 ± 3.10	75.17 ± 2.19	73.33 ± 2.44	76.00 ± 1.24	74.17 ± 2.21	70.33 ± 2.63	-	3.83	6.19	2.77	5.11	10.02
Glibenclamide (5 mg/kg)	77.17 ± 1.17	42.50 ± 0.76***	34.00 ± 0.36***	31.83 ± 0.60***	34.83 ± 0.79***	33.00 ± 0.58***	-	44.92	55.94	58.75	54.75	57.86
Leaf extract (500mg/kg)	80.50 ± 0.76	78.17 ± 3.81	76.83 ± 1.81	69.33 ± 1.85**	68.83 ± 1.19**	65.50 ± 1.80	-	2.89	4.55	13.87	14.49	18.63
Flower extract (500mg/kg)	74.00 ± 0.97	71.00± 0.82	65.00± 0.73*	61.50 ± 1.82***	57.33 ± 0.88**	55.00 ± 1.07***	-	4.05	12.16	16.89	22.52	25.67

n= 6 animals in each group. Values are expressed as Mean ± SEM. *p<0.05, ** p<0.01, ***p<0.001 vs. Normal control

Effect of Jasminum grandiflorum leaf and flower extracts on blood glucose level in glucose-overloaded healthy albino rats

Results of the OGTT test revealed that flower extract had better effect on higher glucose levels than leaf extract (Table 2). Control animals showed a significant rise in the blood glucose levels at 0.5 h and 1 h, which started declining thereafter till the end of the experiment. Flower extract reduced the blood glucose level significantly at 2 and 4 h after glucose load, whereas treatment with leaf extract produced a significant decrease in glucose level only at 4 h compared to control group. Efficacy of the flower extract ($p<0.001$) was almost similar to that of glibenclamide at 4 h ($p<0.001$) and normal control. Flower extract showed 15.46% percent decrease in the glucose level, while the leaf extract reduced the glucose level by 8.45%.

Table 2: Effect of Jasmine grandiflorum leaf and flower extracts extract on blood glucose level in glucose-overloaded healthy albino rats.

Treatment	Fasting blood glucose (mg/dL)					Reduction in blood glucose(%)				
	Time (h)					Time (h)				
	0.0	0.5	1.0	2.0	4.0	0.0	0.5	1.0	2.0	4.0
Normal control	85.33± 2.39	107.17± 2.18	138.00± 4.04	88.00± 2.03	84.50± 3.70	-	- 25.59	- 61.72	- 3.12	- 0.97
Glibenclamide (5 mg/kg)	89.50± 0.76	105.17± 1.42	90.43± 0.87**	80.50± 0.76**	67.50± 0.76***	-	- 17.50	- 1.03	10.05	24.58
Leaf extract (500 mg/kg)	84.83± 4.62	125.33± 2.65	117.33± 3.87	91.33± 4.86	77.66± 2.76*	-	- 47.74	- 38.31	- 7.66	8.45
Flower extract (500 mg/kg)	84.66 ± 3.40	119.66± 1.05	118.66± 1.17	81.69 ± 5.23**	71.57 ± 2.51***	-	- 41.34	- 40.16	3.50	15.46

n= 6 animals in each group. Values are expressed as Mean ± SEM. *p<0.05, ** p<0.01, ***p<0.001 vs. Normal control

Evaluation of antidiabetic potential of leaf and flower extracts of Jasminum grandiflorum in streptozotocin-induced diabetic rats

Effect of Jasminum grandiflorum leaf and flower extracts on fasting mean blood glucose level values before and after the treatment is given in (Table 3). Data from this experiment suggested that compared to diabetic control group, flower extract-treated group was able to significantly reduce blood glucose level in a time-dependent manner from day 7 through day 28 of the treatment period with highest reduction observed at the end of the study ($p<0.001$). Similar effect was seen with standard drug in a time-dependent manner. However, hypoglycemic effect of the leaf extract, though significant, was less compared to that of flower extract at all-time points. When percent reduction was compared, it was found that efficacy of the flower extract was double (68.71%) than the leaf extract (34.87%) and comparable to that of the standard treatment (76.64%).

Table 3: Effect of Jasmimum grandiflorum leaf and flower extracts instreptozotocin-induced diabetic rats.

Treatment	Fasting blood glucose (mg/dL)					Reduction in blood glucose(%)				
		Days					Time (h)			
	0	7	14	21	28	0	7	14	21	28
Normal control	80.50± 7.67	81.16± 0.60	80.83± 0.60	80.67± 0.67	80.67± 0.67	-	- 0.81	- 0.40	- 0.21	- 0.21
Diabetic control	393.67± 2.76	367.50± 2.70	358.83± 2.23	355.17± 2.12	353.67± 1.70	-	6.60	8.84	9.70	10.16
Glibenclamide (5 mg/kg)	394.67± 2.47	207.33± 2.49***	177.50± 2.26***	110.17± 1.85***	92.17± 2.49***	-	47.46	55.02	72.08	76.66
Leaf extract (500mg/kg)	397.67± 1.47	359.50± 2.81	275.50± 2.81*	267.00± 1.90**	259.00± 1.71**	-	9.59	30.72	32.85	34.87
Flower extract (500mg/kg)	417.17± 2.77	258.67± 2.61**	197.67± 2.17***	147.33± 1.358***	130.50± 1.48***	-	37.99	52.61	64.68	68.71

n= 6 animals in each group. Values are expressed as Mean ± SEM. *p<0.05, ** p<0.01, ***p<0.001 vs. Diabetic control

Effect of leaf and flower extracts of Jasminum grandiflorum on pancreas in streptozotocin-induced diabetic rats

Histopathology studies of the experimental rats were measured after 28 days of treatment. The pancreas of normal control group showed normal appearance of islet of Langerhans containing α, β, and δ cells. The β cells were the most abundant cells (Figure 1a). Diabetic control group showed reduction in the pancreatic β cell numbers, β cell vacuolization, and necrosis, as well as a few surviving β cells. In addition, severe congestion of pancreatic parenchyma and infiltration of inflammatory cells was observed. A marked degeneration of the islet of Langerhans with severe vacuolization of the exocrine tissue was also observed (Figure 1b). However, in case of glibenclamide-treated group, pancreas architecture was similar to that observed in the control rats with slight hyperplasia of islets cell (Figure 1c). Histopathological examination of pancreas of STZ-induced diabetic rats treated with leaf extract of Jasminum grandiflorum revealed no significant difference when compared with diabetic control group (Figure 1d). Whereas, the group treated with the flower extract showed a significant reduction in the extent of necrosis, vacuolization, and increase in the number of β islet cells of pancreas (Figure 1e), which was found to be far better than the group treated with standard drug glibenclamide.

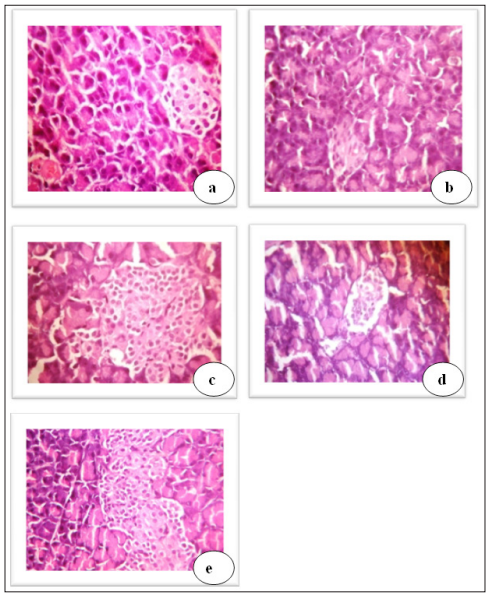

Figure 1: Effect of Jasmimum grandiflorum leaf and flower extracts on pancreas histopathology in streptozotocin-induced diabetic rats. (a) Normal control (b) diabetic control (c) standard (glibenclamide) (d) leaf extract-treated (500 mg/kg, p.o.) (e) flower extract-treated (500 mg/kg, p.o.).

Evaluation of antidiabetic activity of different fractions of flower extract of Jasminum grandiflorum

Owing to significant blood glucose-lowering efficacy of the flower extract, fractionalization of the same was carried out using four different solvents, viz. petroleum ether, ethyl acetate, methanol and chloroform. These concentrated fractions were further evaluated for their antidiabetic activity in streptozotocin model of diabetes. Effect of different fractions of ethanol extract of Jasminum grandiflorum flowers on the blood glucose level is presented in (Table 4).

Table 4: Effect of ethanol extract of Jasmimum grandiflorum flower fractions in streptozotocin-induced diabetic rats.

Treatment	Fasting blood glucose (mg/dL)					Reduction in blood glucose (%)				
	Days					Time (h)				
	0	7	14	21	28	0	7	14	21	28
Diabetic control	393.67 ± 2.76	367.50 ± 2.70	358.83 ± 2.23	355.17 ± 2.12	353.67 ± 1.70	-	6.60	8.84	9.70	10.16
Glibenclamide (5 mg/kg)	394.67 ± 2.47	207.33 ± 2.49***	177.50 ± 2.26***	110.17 ± 1.85***	92.17 ± 2.49***	-	47.46	55.02	72.08	76.66
Petroleum ether fraction (200 mg/kg)	323.00 ± 10.50	268.00 ± 12.50**	255.00± 10.60***	223.00± 8.82***	200.00 ± 6.83***	-	17.02	21.07	30.97	38.08
Ethyl acetate fraction (200 mg/kg)	315.00 ± 8.47	295.00 ± 8.53	272.00 ± 2.95**	236.00 ± 9.17***	195.00 ± 4.28***	-	6.34	13.65	25.07	38.09
Methanol fraction (200 mg/kg)	303.00 ± 11.50	278.00 ± 9.46	251.00 ± 3.00***	204.00 ± 4.55***	182.00 ± 2.08***	-	8.25	17.16	32.67	39.93
Chloroform fraction (200 mg/kg)	317.00 ± 5.58	291.00 ± 2.71	252.00 ± 15.60***	185.00 ± 3.93***	145.20 ± 1.01***	-	8.20	20.50	41.64	54.29

n= 6 animals in each group. Values are expressed as Mean ± SEM. *p<0.05, ** p<0.01, ***p<0.001 vs. Diabetic control

Streptozotocin-induced diabetic rats were treated with different fractions of ethanol extract of Jasminum grandiflorum flowers once daily for

28 days to evaluate the long-term efficacy. Data clearly demonstrated that all four fractions were able to reduce blood glucose level in a time-dependent manner at 200 mg/kg dose. While petroleum ether fraction significantly reduced the glucose levels at all-time points (i.e. on day 7, 14, 21, and 28), other three fractions (i.e. ethyl acetate, methanol, and chloroform fractions) produced anti-hyperglycemic effects from day 14 onwards and maintained till the end of the experiment. When percentage reduction in blood glucose level was calculated, it was observed that chloroform fraction was more potent amongst all four fractions, showing 54.29% decrease in blood glucose level by the end of the experiment. Standard treatment led to significant hypoglycemic effect at time points ($p<0.001$) when compared to diabetic control group. Similarly, glibenclamide produced 76.66% reduction in blood glucose level than diabetic control.

Evaluation of cytotoxicity of the chloroform isolate

Based on the encouraging results obtained from chloroform fraction, we further evaluated cytotoxicity of chloroform isolate from the ethanol extract of Jasminum grandiflorum flowers against L6 cell line at different concentrations to determine the IC_{50} value. Test results demonstrated that the cytotoxicity of the chloroform was found to be dose-dependent with 65.28 ± 0.9 % cell toxicity at 50 µg/ml concentration (Table 5).

Table 5: In vitro cytotoxicity evaluation of chloroform isolate from Jasmimum grandiflorum flower extract using MTT Assay.

Sample	Concentration (µg/ml)	Cytotoxicity (%)	CTC_{50} (µg/ml)
Jasmimum grandiflorum (chloroform isolate)	50	65.28 ± 0.9	17.52 ± 1.9
	25	58.37 ± 2.7	
	12.5	44.49 ± 2.1	
	6.25	25.20 ± 3.8	
	3.12	17.32 ± 1.7	
	1.56	5.35 ± 1.7	

Glucose uptake activity

Based on the CTC_{50} value (17.52 ± 1.9%) the concentration of the test substance was considered for glucose uptake activity in L6 cell line and results are presented in (Table 6). The glucose utilization in L6 cell line showed that the chloroform isolate was significantly higher over control. The L6 cell line enhanced the glucose uptake by 60.07 ± 2.37% and 29.36 ± 1.12%at 10 and 5 µg/ml concentration, respectively. While rosiglitazone, a standard, at 100 µg/ml concentration found to enhance the glucose uptake by 124.13 ±2.39% when compared to control.

Table 6: Influence of chloroform isolate from Jasmimum grandiflorum flower extract in L6 cell line.

Sample	Concentration (µg/ml)	Protein content	Glucose uptake (%)
Control	-	1004.50	0.20 ± 0.15
Dimethyl sulfoxide (DMSO) Control	-	1004.32	0.09 ± 0.69
Rosiglitazone	100	1010.40	124.13 ± 2.39
Jasmimum grandiflorum (chloroform isolate)	10	1057.62	60.07 ± 2.37
	5	991.01	29. 36 ± 1.12

Discussion

Diabetes mellitus has become a global health challenge. Multiple pathogenetic/heterogeneous nature of the disease warrants the need for more efficacious and safe therapeutic options. Several factors contribute to the progression of diabetes, including hyperglycemia and individual components of the metabolic syndrome along with environmental and genetic factors. All these factors would lead to multiple pathophysiological disturbances, such as micro vascular and macro vascular complications [36, 37].

In the present study, we have attempted to establish pharmacological significance of Jasminum grandiflorum in the treatment of diabetes, hypoglycemic potential of different fractions of flower extract in normoglycemic rats, glucose-overloaded rats and streptozotocin model of diabetes in rats. Additionally, chloroform isolate from ethanol extract of flowers of Jasminum grandiflorum was also studied for antidiabetic efficacy in vitro. Data from the present study indicated that ethanol extract of Jasminum grandiflorum flowers and different fractions produced significant anti-hyperglycemic effects in vivo, while chloroform isolate was found to be cytotoxic.

Streptozotocin, a well-known diabetogenic agent in diabetes research, induces a state of insulin-dependent diabetes by modifying biological macromolecules, DNA fragmentation, and destructing the beta cells [38]. From the above results, the ethanol extract of Jasminum grandiflorum flowers and its various fractions were found to be more potent in the reducing the blood glucose level in diabetic animals under pathophysiological condition. Therefore, Jasminum grandiflorum may be involved in stimulating the insulin secretion and pancreatic β cell regeneration or increasing cellularity of the islet tissue and regeneration of the granules in the β-cells and/or protection against necrosis. Hence, based upon the results it can be hypothesized that the flower extract and different fractions declined the level of the blood glucose and improved the pancreas insulin level [39]. Additionally, histopathological evaluation of the pancreas further ascertained this hypothesis, wherein the flower extract showed significant protection of the pancreas from the toxic effect of microbial streptozotocin through reduced extent of necrosis, vacuolization, and increased number of β islet cells.

The phytochemical studies revealed the presence of essential oil, tannins, triterpenoids, flavonoids, and phenolic compounds in the flower extract. Based on the current study outcomes as well as previously reported data we hypothesize that the antidiabetic activity of Jasminum grandiflorum flower extract may be due to the presence of essential oil, tannins, triterpenoids, flavonoids, and phenolic compounds, which are believed to enhance insulin secretion and also improve the antioxidant defense [37, 41-43]. Lower hypoglycemic efficacy of the leaf extract may possibly be due to the absence or lack of such antidiabetic metabolites. Results of in vitro glucose uptake activity suggested that the chloroform isolate enhanced the glucose uptake, which further corroborates our hypothesis of antidiabetic activity of phytoconstituents present in Jasminum grandiflorum. However, the chloroform isolated from Jasminum grandiflorum produced cytotoxicity in L6 cell line and at present we are unable to ascertain any possible reason behind this effect, which we believe warrants additional experiments.

Conclusion

Based on the toxicity data, it appears that Jasminum grandiflorum extract is safe with no possible side effects even at higher dosage.

Overall results of the study therefore advocate that Jasminum grandiflorum could be a potential candidate in the treatment of diabetes mellitus. Although the present results do provide a potential explanation by which Jasminum grandiflorum exerts desired therapeutic effect, further research is required to confirm this hypothesis.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgment

The authors thank Rajiv Gandhi University of Health Sciences (RGUHS), Bengaluru, Karnataka, India, for sanctioning research grant for this work.

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