IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 11 Ver. III (Nov. 2017), PP 08-13
www.iosrjournals.org

Anti-Diabetic Properties of The Methanolic Leaf Extract Of *Bougainvillea Glabra* On Alloxan-Induced Diabetic Rats

*Dr. Sam Pavan Kumar G, Govardhan Reddy Kasireddy, Dr. Anil C, D. Sathish Kumar, Eppili Bhavani

Associate Professor, Department of Pharmacology, Chalmeda Anandarao Institute of Medical Sciences, Hyderabad, Telangana.
*Corrsponding Author: *Dr. Sam Pavan Kumar*

Abstract: *The antidiabetic and antilipidemic effects of Bougainvillea glabra was investigated in this study using 25 male wistar rats. The rats were divided into 5 groups comprising of five animals each. These groups include Group I: Normal control (saline).Group II: Alloxan treated control (150 mg/kg.ip).Group III: Alloxan (150 mg/kg.ip) + Bougainvillea glabra. Whole plants extract (300 mg/kg, p.o),Group IV: Alloxan (150 mg/kg.ip) + Bougainvillea glabra. Whole plants extract (500mg/kg, p.o),Group V: Alloxan (150 mg/kg.ip) + Standard drug, Glibenclamide (5 mg/kg, p.o). The study lasted for three weeks although blood samples were obtained from the rat tails after every week. The results show that the extract significantly (p< 0.05) reduced the hyperglycaemia from 12±0.40 mmol/L (Diabetic Control) to 4.04±0.03 mmol/L (400 mg/kg group). Likewise, the extract significantly reduced the Total Cholesterol (TC), Triglyceride (TG) and Low-Density Lipoprotein Cholesterol (LDL-Cholesterol), while increasing the High-Density Lipoprotein Cholesterol (HDL-C). In conclusion, the observations from this study show that Bougainvillea glabra has antidiabetic effect and beneficial effects on blood lipid profile, thus justifying the use of the plant by traditional medicine practitioners for the treatment of diabetes mellitus.*

Keywords: *Bougainvillea glabra, alloxan-diabetes, hypoglycaemia, hypolipidemia.*

Date of Submission: 26 -10-2017 Date of acceptance: 09-11-2017

I. Introduction

Diabetes mellitus is one of the common metabolic disorders with micro-and macrovascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world [1, 2]. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus [3]. There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents [4–6]. There are numerous traditional medicinal plants reported to have hypoglycemic properties such as Allium sativum (Garlic), Azadirachta indica (Neem), Bougainvillea glabra (Nayantara), Trigonella foenum (Fenugreek), Momordica charantia (Bitter ground), Ocimum santum (Tulsi). Many of these are less effective in lowering glucose levels in severe diabetes.

Bougainvillea (with the common name Glory of the Garden), originated from South America and it is a popular plant in Southern California, Florida, the Caribbean and other areas with tropical and warm climates [8]. It is a thorny woody plant with flowers ranging from pink, purple, red, orange, yellow colours and especially white [9,10]. Among the Bougainvillea's varieties include *Bougainvillea glabra*, *Bougainvillea spectabilis*, and *Bougainvillea harrisi*. *Bougainvillea glabra* is grown for its decorative purposes in tropical regions and in the temperate [8]. In some areas like the Western Europe, North Africa, Middle East and the Indian Sub-continent, *B. Spectabilis*, is used to shorten the life span of sand flies that cause leishmaniasis [11]. *Bougainvillea spectabilis* is also used in herbal combination for the treatment of diabetes [12]. Information also exist about the use of *Bougainvillea glabra* in the cure of ulcer, diarrhoea, and having anti-microbial activities[13]. Information from some traditional medical practitioners show that some beetles that feed on any *Bougainvillea* stems are dried, crushed into powder and added as main ingredient in popular herbal combinations used in the treatment of diabetes mellitus. These herbal formulations are said to be incomplete without the *Bougainvillea* stem beetle-fed ground powder[14].

This study was designed to examine the hypoglycemic and antilipidemic effects of *Bougainvillea glabra* another *Bougainvillea* species apart from B. *spectabilis* on alloxan-induced Diabetes Mellitus based on the local uses of the plant for the treatment of diabetes mellitus.

II. Material and Methods

2.1. Plant Material

The basic plant material of *Bougainvillea glabra* whole plant used for the investigation was obtain from Mount Opera Garden, Near Ramoji Film City, Nalgonda Dist, Andhra Pradesh, India. The plant can be identified authenticated by department of Botany research office (Botanist), Karimnagar.

2.2. Alcoholic Extraction

The whole plants were collected and shadow dried. The shade-dried whole plants were subjected to pulverization to get coarse powder. The coarsely powder whole plant (1 kg) of Bougainvillea glabra was used for extraction with methanol in soxlate apparatus. The extract was evaporated to dryness under vacuum and dried in vacuum desiccator (15.5% w/w).

2.3. Animals

Wistar albino rats (8–10 weeks) of both sexes were obtained from the animal house of Nizam Institute Of Pharmacy, Deshmukhi, Ramoji Film City, Hyderabad. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours ad libitum.

2.4. Oral Glucose Tolerance Test

Rats were divided into six groups containing six animals in each group. All animals fasted before treatment. Group I was kept as vehicle control which received 5% Tween 80 p.o., group II received glucose only, group III received methanolic extract 300 mg/kg, group IV received methanolic extract 500 mg/kg and group V and VI received only extracts (300 mg/kg and 500 mg/kg) only in a vehicle, respectively.The rats of group III and IV were loaded with glucose (3 g/kg, p.o.) 30 minutes after drug administration. Blood samples were collected from puncturing the retro orbital sinus just prior to drug administration, and 30, 90, 150 minutes after loading glucose. Serum glucose level was measured immediately by using glucose estimation kit (Span Diagnostic Pvt. Ltd. Surat, India).

2.5. Acute Oral Toxicity Studies

Bougainvillea glabra at the dose range of 100 mg–2000 mg/kg were administered orally to different group of rats comprised of ten rats in each group. Mortality was observed after 72 hours. Acute toxicity was determined according to the method of Litchfield and Wilcoxon [14].

2.6. Experimental Design

Five groups of rats, six in each received the following treatment schedule. Group I: Normal control (saline). Group II: Alloxan treated control (150 mg/kg.ip). Group III: Alloxan (150 mg/kg.ip) + *Bougainvillea glabra*. Whole plants extract (300 mg/kg, p.o), Group IV: Alloxan (150 mg/kg.ip) + *Bougainvillea glabra*. Whole plants extract (500mg/kg, p.o), Group V: Alloxan (150 mg/kg.ip) + Standard drug, Glibenclamide (5 mg/kg, p.o).

Whole plant extracts and standard drug glibenclamide (5 mg/kg) and saline were administered with the help of feeding cannula. Group I serve as normal control, which received saline for 14 days. Group II to Group V are diabetic control rats. Group III to Group V (which previously received alloxan) are given a fixed dose whole plants extract (300 mg/kg, p.o), (500 mg/kg, p.o) and standard drug glibenclamide (5 mg/kg) for 14 consecutive days.

2.7. Induction of Diabetes in Experimental Animals

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg) [15]. Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of 140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection.

2.8. Collection of Blood Sample and Blood Glucose Determination

Blood samples were drawn from tail tip of rat at weekly intervals till the end of study (i.e., 2 weeks). Fasting blood glucose estimation and body weight measurement were done on day 1, 7, and 14 of the study. Blood glucose estimation can be done by one touch electronic glucometer using glucose test strips.

On day 14, blood was collected from retro-orbital plexus under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated [16]. Serum was separated and analyzed for serum cholesterol [17], serum triglycerides by enzymatic DHBS colorimetric method [18], serum HDL [19], serum LDL [20], serum creatinine [21], serum urea [22] and serum alkaline phosphatase hydrolyzed phenol amino antipyrine method [23] was estimated. The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formaline solution, and immediately processed by the paraffin technique. Sections of 5thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination.

2.9. Statistical Analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's -test. Differences between groups were considered significant at $P < .01$ levels.

III. Results

3.1. Glucose Tolerance

The effects of extracts of *Bougainvillea glabra* (500 mg/kg and 300 mg/kg) on glucose tolerance test are shown in Figure 1. The supplementation of *Bougainvillea glabra* improved the glucose tolerance in the fasted normal rats. After that serum glucose level was lowered significantly ($P < .05$) at 90 minutes and varied significantly ($P < .01$) lowered at 150 minutes. Extract also showed significant hypoglycemic effect after 90 minutes of treatment.

3.2. Experimental Results

The acute oral toxicity study of *Bougainvillea glabra* showed no mortality upto 2000 mg/kg. The anti-hyperglycemic effect of the extracts on the fasting blood sugar levels of diabetic rats is shown in Figure 3. Administration of alloxan (150 mg/kg, i.p.) lead to 1.5-fold elevation of fasting blood glucose levels, which was maintained over a period of 2 weeks. Two weeks of daily treatment of various extract of Bougainvillea glabralead to a dose-dependent fall in blood sugar levels by 25%–50%. Effect was maximum till 14 days of treatment. Vehicle control animals were found to be slightly increased in their body weight but diabetic rats showed significant reduction in body weight during 14 days (Figure 3). Alloxan caused body weight reduction, which is reversed by whole plant extract at high dose (500 mg/kg) is more effectively than whole plant extract at low dose (300 mg/kg) after 14 days of treatment (Figure 4). Alloxan treatment will increase the serum enzymes levels such as cholesterol,LDL, creatinine, urea and alkaline phosphatase and decrease the HDL level, but glibenclamide (5 mg/kg) and whole plant extracts of Bougainvillea glabrareversed the above alloxan induce changes (Table 1). Histopathological studies showed normal acini and normal cellular population in the islets of Langerhans in pancreas of control rats (Group I). Extensive damage to the islets of Langerhans and reduced dimensions of islets (Group II), restoration of normal cellular population size of islets with hyperplasia by glibenclamide (Group V) were also shown. The partial restoration of normal cellular population and enlarged size of -cells with hyperplasia were shown by methanolic extracts .

Table 1: Effect of various groups of Bougainvillea glabra on serum profile in alloxan (150 mg/kg, i.p.) induced diabetic albino rats after 14 days of treatment.

S.No	Groups	Cholesterol (mg/dl)	H.D.L (mg/dl)	L.D.L (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Alkaline Phosphatase (mg/dl)
1	Normal control	146.36 ± 3.2	36.83 ± 2.5	91.32 ± 1.2	0.54 ± 0.3	31.83 ± 2.2	120 ± 3.2
2	Diabetic control	272.16 ± 10.5	30.00 ± 1.9	189 12.4	2.4 ± 0.1	62.6 ± 1.8	276.00 ± 3.6
3	Alloxan + Whole plant extract (300 mg/kg,p.o)	184.32 ± 2.5*	34.22 ± 4.3*	120.27 ± 1.4*	0.98 ± 0.3*	43.32 ± 3.8*	146.35 ± 4.9*
4	Alloxan + Whole plant extract (500 mg/kg,p.o)	158.46 ± 5.6*	36.63 ± 2.1*	93.65 ± 3.6*	0.60 ± 0.2*	32.33 ± 2.0*	135.55 ± 4.9*
5	Alloxan + glibenclamide (5 mg/kg)	145.42 5.3*	36.73 ± 1.5	92.35 ± 3.1*	0.58 ± 0.1*	31.244.0*	130.75 ± 2.9*

Values are given as mean ± SEM for groups of six animals each *$P < .01$ (Dunnet -test). Diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control.

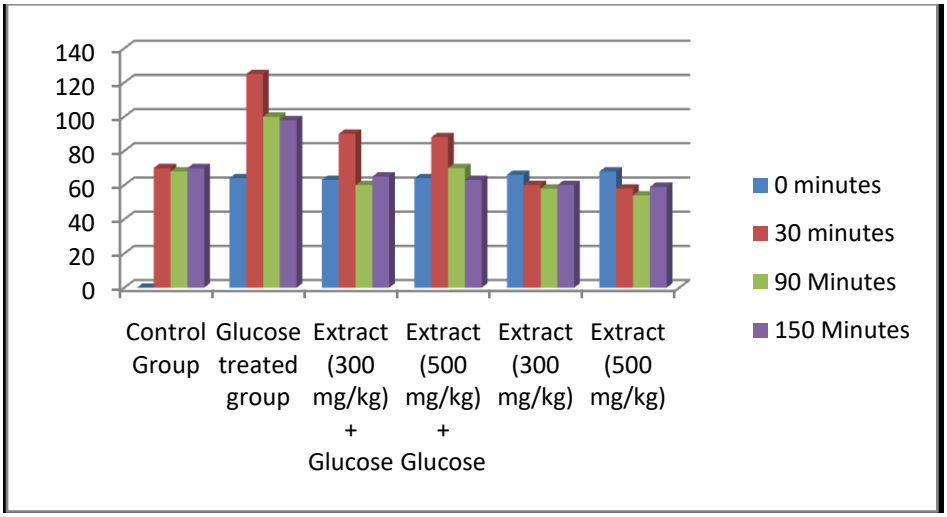

Figure 1: Effect of methanolic extract of Bougainvillea glabra on Glucose tolerance test

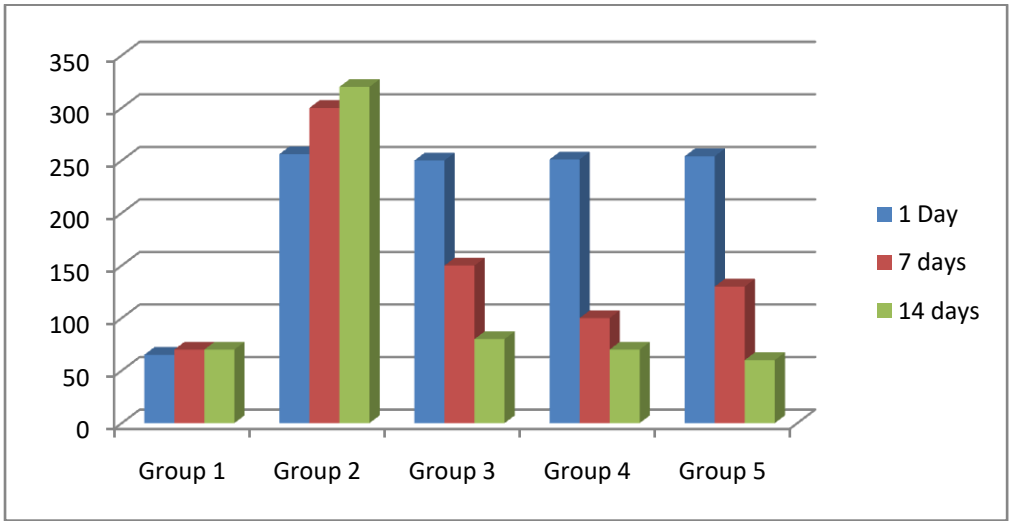

Figure 2: Effect of different groups on blood glucose (mg/dl) level in alloxan induced diabetes

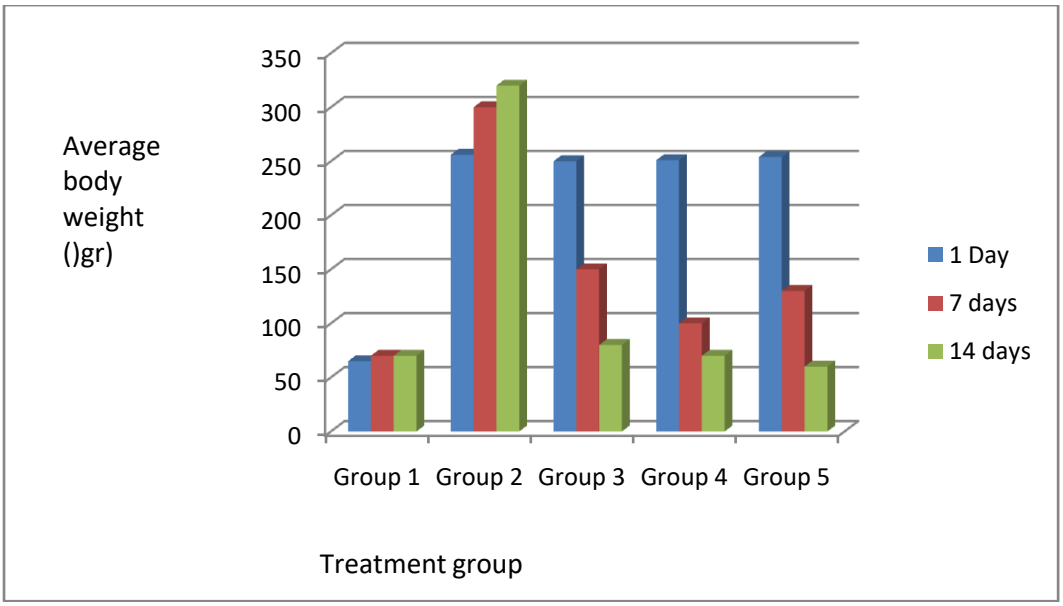

Figure 3: The effect of 2-week treatment with various extracts of *Bougainvillea glabra* on body weight (g) after alloxan (150 mg/kg i.p.) induced diabetes in rats.

IV. Conclusion

In light of the results, our study indicates that methanolic extracts of Bougainvillea glabrahave good antidiabetic activity. Alcoholic extracts of Bougainvillea glabraexhibited significant anti-hyperglycemic activities in alloxan-induced hyperglycemic rats without significant change in body weight; they can also improve the condition of Diabetic mellitus as indicated by parameters like body weight & lipid profile along with serum creatinine, serum urea and serum alkaline phosphatase. The renewal of cells in diabetes have been studied in several animal models. The total cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet ß cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug [24]. Bougainvillea glabrawhole plant alcoholic extracts has been shown to act by cell regeneration. Similar effects in streptozotocin-treated diabetic animals were reported by pancreas tonic [25], ephedrine [26], and Gymnema sylvestre leaf extracts [27]. In our studies, the damage of pancreas in alloxan-treated diabetic control rats (Figure 1 Group II) and regeneration of cells by glibenclamide (Figure 1 Group V) was observed. It is found that methanolic whole plant extract at high dose (500 mg/kg) is more effective than whole plant extract at low dose (300 mg/kg) after 14 days of treatment. Hence the above discussion revels that methanolic whole plant extract at high dose (500 mg/kg) is more effective and shows similar curative effect as standard that is, glibenclamide (5 mg/kg). This could be due to the possibility that some -cells are still surviving to act upon by Bougainvillea glabraextract to exert its insulin releasing effect. Histopathological studies reinforce the healing of pancreas, by Bougainvillea glabraextracts, as a possible mechanism of their antidiabetic activity.

Conclusion

The whole plant extracts did not show a consistent effect on normal blood sugar levels but it effectively reversed the alloxan-induced changes in the blood sugar level and the beta-cell population in the pancreas. It also showed a protective effect when it was given prior to alloxan administration. The action of whole plant extracts on the pancreatic beta-cells and absence of acute toxicity may offer a new hope to the diabetics in future.

From the above discussion it conclude that alcoholic whole plant extracts of Bougainvillea glabra at high dose (500 mg/kg) exhibited significant antihyperglycemic activity than whole plant extract at low dose (300 mg/kg) in alloxan-induced diabetic rats. These extracts also showed improvement in parameters like body weight and lipid profile as well as regeneration of cells of pancreas and so might be of value in diabetes treatment. Further investigation is in necessary to determine the exact phytoconstituents (s) responsible for antidiabetic effect.

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Dr. Sam Pavan Kumar G Anti-Diabetic Properties of The Methanolic Leaf Extract Of Bougainvillea Glabra On Alloxan-Induced Diabetic Rats." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 16.11 (2017): 08-13.