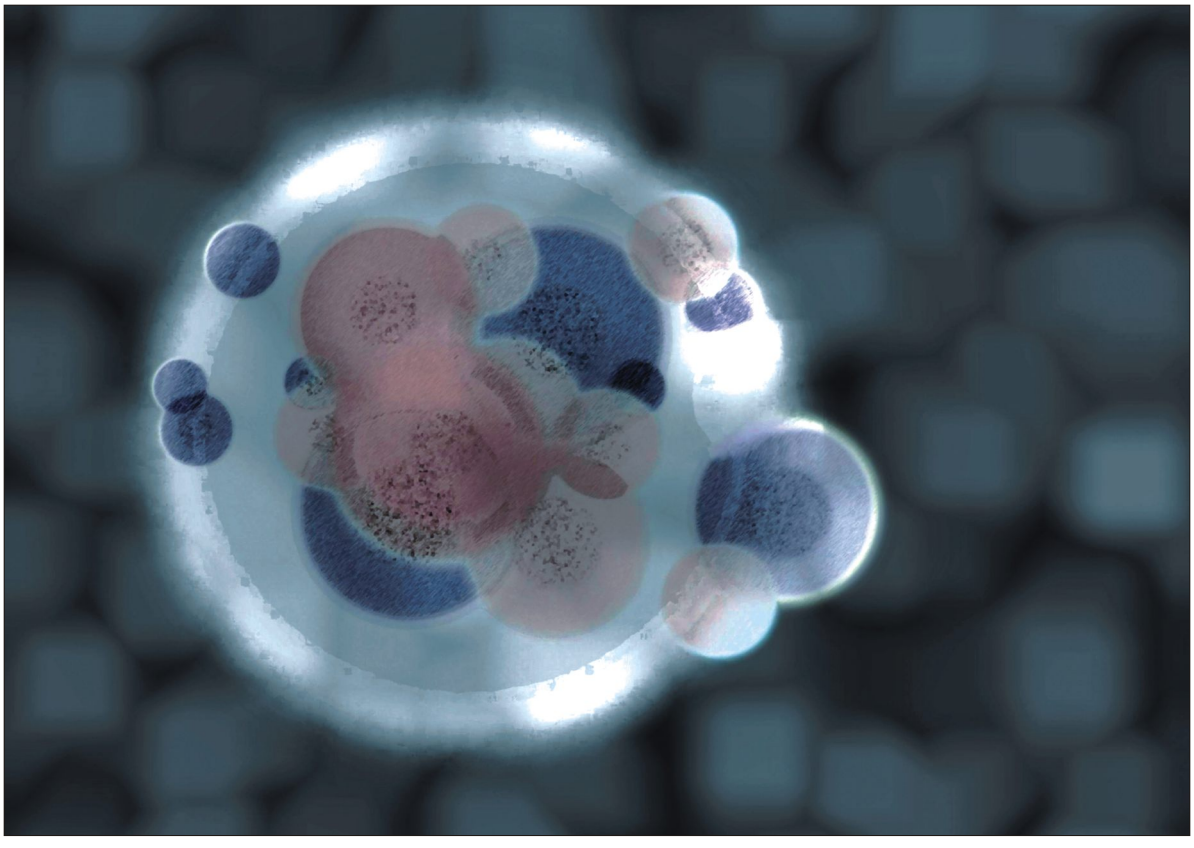

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Research Article
Caesalpinia pulcherrima Extracts on Blood Glucose in Normal and Alloxan Monohydrate-induced Diabetic Rats

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Abstract

Background and Objective: Type 2 Diabetes Mellitus is a long term endocrine metabolic disorder that is described by elevated blood sugar, insulin resistance and deficiency of insulin. Due to low toxicities and cost effectiveness natural products are comparatively safe and good source of effective antidiabetic agents. The present study designed to evaluate the effects of *Caesalpinia pulcherrima* extracts on blood glucose in normal and alloxan monohydrate-induced diabetic rats. **Materials and Methods:** Albino rats of Wistar strain (150-200 g) of either sex were used in the entire study. 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 for 16 h and water *ad libitum*. Sample collection-blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated using an electronic glucometer. **Results:** Three weeks of daily treatment of Alloxan induced methanolic extract of *Caesalpinia pulcherrima* at dose 400 mg kg^{-1} led to a dose dependent fall in blood sugar levels from 290-233 mg dL^{-1} in 21 days (Group 6). **Conclusion:** Extracts of pods of *C. pulcherrima* are capable of exhibiting reduction in blood glucose level in normal and alloxan monohydrate-induced diabetic rats.

Key words: *Caesalpinia pulcherrima*, blood glucose, retro-orbital plexus puncture method, alloxan, diabetes mellitus and antidiabetic agents

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus is not only a chronic illness associated with substantial morbidity and mortality but also a major public health problem of epidemic proportions, with a projected prevalence of 300 million cases worldwide[1] by 2025. The rapidly escalating prevalence of diabetes is related to several factors, including the alarming increase in obesity[2], mainly due to sedentary lifestyle and high-fat diets and the aging population. More than 85% of individuals with diabetes mellitus have type 2 diabetes and the lifetime risk of developing this disorder approaches 20% in the United States[3]. Results from the 3rd National Health and Nutrition Examination Survey estimated the prevalence of type 2 diabetes in adults (>20 years) at 5.1%, undiagnosed cases at 2.7% and impaired glucose tolerance (IGT) at 6.9% (based on fasting plasma glucose values)[4]. These statistics are especially disturbing in light of the fact that diabetes is the leading cause of new cases of blindness, end-stage renal disease and lower extremity amputation[5] and with nearly 50% of diabetes-related mortality being caused by cardiovascular disease, contributes substantially to cardiovascular death in the United States[6]. Current patient self management education and support are critical to preventing acute complications and reducing the danger of long-term complications. Considerable confirmation exists that supports a variety of involvement to improve diabetes outcomes[7,8]. The long-term complications associated with diabetes (including micro vascular and macro vascular diseases) have been reviewed extensively[9-12]. Diabetes mellitus may be categorized into several types but the two major types[13] are type 1 and type 2. On the basis of etiology, the term type 1 and type 2 were widely used to describe IDDM and NIDDM, respectively. The term juvenile-onset diabetes has sometimes been used for IDDM and maturity-onset for NIDDM. On the basis of etiology, type 1 is present in patients who have little or no endogenous insulin secretory capacity and who therefore require insulin therapy for survival. Type 1a is characterized by the presence of islet cell antibody (ICA), anti-glutamic acid decarboxylate (anti-GAD), IA-2 or insulin antibodies that identify the autoimmune process with β-cell destruction[14]. There is no known etiological basis for type 1b diabetes mellitus. Some of these patients have permanent insulinopaenia and are prone to ketoacidosis, but have no evidence of autoimmunity[15]. Type 2 diabetes is the commonest form of diabetes and is characterized by disorders of insulin secretion and insulin resistance. The disease is usually controlled through dietary therapy, exercise and hypoglycaemic agents[16,17]. *Caesalpinia pulcherrima* belonging to the family Caesalpiniaceae is an ornamental plant widely used for the treatment of various ailments across India[18]. Phytochemical investigations on *Caesalpinia pulcherrima* have revealed the presence of various phytoactive constituents such as glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, flavones and sterols[19,20]. *Caesalpinia pulcherrima* L. *Swartz* (Caesalpiniaceae) is an ornamental plant due to its variety of flowers, which appear yellow, pink, off-white and red with yellow margins[21]. Its seeds have shown antiviral activity[20] stem shown cytotoxic activity[22], leaves shown antitumor activity[20], antimicrobial activity[23], antiviral activity[20] , flowers shown antimicrobial activity[24], fruits shown antiviral activity[20], bark shown antimicrobial, cytotoxic[25]. Kumbhare and Sivakumar[26] evaluated anti-inflammatory activity of various extracts of pods of *Caesalpinia pulcherrima* using the Carrageenan induced rat paw oedema. Kumbhare *et al.*[27] evaluated *in vitro* antioxidant activity by DPPH free radical scavenging assay and by nitrous oxide free radical scavenging assay of methanol, chloroform and petroleum ether extracts of Pods of *Caesalpinia pulcherrima*. Kumbhare *et al.*[27] evaluated cytotoxic activity in brine shrimp lethality bioassay of crude petroleum ether, chloroform and methanolic extract of pods of *Caesalpinia pulcherrima* showed lethality against the brine shrimp nauplii. Kumbhare *et al.*[28] evaluated *in vitro* anthelmintic potency of petroleum ether, Chloroform and Methanolic extracts of pods of *Caesalpinia pulcherrima* using Indian earthworms (*Pheretima posthuma*).

The present study sought to determine the effects of extracts CP on blood glucose in normal and alloxan monohydrate-induced diabetic rats. For these analyses, a well-known model, i.e., the alloxan induced diabetic rat, was employed.

MATERIALS AND METHODS

Plant material: Pods of *Caesalpinia pulcherrima* Fam. Caesalpiniaceae were collected from local region of Nashik, India in October 2008. The plant material was identified and authenticated by Dr. P.G. Diwakar Botanical survey of India, Pune (Ref. No. BSI/WC/Tech/2009/370).

Preparation of extract: The plant material were cleaned, dried under shade and pulverized by using grinder. About 500 g of the powder of plant was successively extracted with petroleum ether, chloroform and methanol in order of their increasing polarity using Soxhlet apparatus. The yield of extracts as follows. The extracts were obtained for *Caesalpinia pulcherrima* petroleum ether as 1.21%, chloroform as 2.46%,

Methanol as 13.32%. From the preliminary Phytochemical study revealed that presence of sterols, glycosides, alkaloids, triterpenoids, flavonoids and tannins in the extracts.

Experimental design and collection of samples: Albino rats of Wistar strain (150-200 g) of either sex were used in the entire study and were procured from Haffkine Institute, Mumbai. They were housed in standard polypropylene cages and kept under controlled room temperature (24±2°C; relative humidity 60-70%) in a 12 h light-dark cycle. The animals were fed with standard laboratory diet of Pranav agro pvt. Ltd. and water *ad libitum*. Food was withdrawn 12 h before and during the experimental hours. The experimental protocol was approved by Institutional Animal Ethical Committee. Wistar albino male rats (150-200 g) were used. Before and during the experiment, rats were fed with standard diet (Pranav Agro, Sangali, 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 for 16 h and water *ad libitum*. Sample collection-blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated using an electronic glucometer[29-31]. The experimental protocol was approved by Institutional Animal Ethical Committee. CPCSEA Reg. No. 1329/PO/Re/S/10/ CPCSEA w.e. 27/10/2016

All the animals were randomly divided into the 6 groups with 6 animals in each group:

- Group I served as normal saline
- Group II diabetic (control) vehicle
- Group III standard drug (glibenclamide, 10 mg kg^{-1} per day p.o.)
- Groups IV, V and VI were treated with methanol extracts of *Caesalpinia pulcherrima* of in doses of 100, 200 and 400 mg kg^{-1} orally respectively per day p.o. Assessment of extracts on alloxan-induced diabetic animals rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (RLCC, Bombay; 150 mg kg^{-1}). Alloxan was first weighed individually for each animal according to the 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^{-1} were included in the study. Treatment with plant extracts was started 48 h after alloxan injection. Blood samples were drawn at weekly intervals till the end of study (i.e. 3 weeks). Fasting blood glucose estimation and body weight measurement were done on day 1, 7 and 21 of the study. On day 21, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and the fasting blood sugar was estimated[32]

RESULTS

Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas. *In vitro* studies have shown that alloxan was selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis. Administration of alloxan (150 mg kg^{-1}, i.p.) led to 1.5-fold elevation of fasting blood glucose levels, which was maintained over a period of 3 weeks.

Three weeks observation in normal control rats blood sugar levels remains as normal (for group 1). Three weeks observation in diabetic control (Vehicle) rats sugar levels remains as from 296-295 mg dL^{-1} (for group 2). Three weeks observation in alloxan+glibenclamide (10 mg kg^{-1}) used as standard fall in blood sugar levels from 295-149 mg dL^{-1} (for group 3). Three weeks observation in Alloxan+Methanolic extract of *Caesalpinia pulcherrima* (100 mg kg^{-1}) fall in blood sugar levels from 294-263 mg dL^{-1} (for group 4).

Three weeks observation in Alloxan + Methanolic extract of *Caesalpinia pulcherrima* (200 mg kg^{-1}) fall in blood sugar levels from 295-242 mg dL^{-1} (for group 5).

Three weeks of daily treatment of extract of *Caesalpinia pulcherrima* led to a dose dependent fall in blood sugar levels from 290-233 mg dL^{-1} in 21 days for methanolic extract of *Caesalpinia pulcherrima* (for group 6) (Table 1).

Effect seems to reach maximum after 15 days of treatment and remains constant in 3rd week. Vehicle control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 21 days. Alloxan caused body weight reduction.

DISCUSSION

The plant extracts showed significant improvement in glucose tolerance in glucose fed hyperglycemic normal rats. Such an effect may be accounted for, in part, by a decrease in the rate of intestinal glucose absorption, achieved by an extra pancreatic action including the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process with concomitant decrease in glycogenolysis and gluconeogenesis. However, the effect was less significant when compared to standard drug glibenclamide. Alloxan is the most commonly employed agent for the induction of

Table 1: Antidiabetic effect of extracts of *C. pulcherrima*

Treatments	Fasting blood glucose level (mg dL^{-1})			
	Basal value	7th Day	14th Day	21st Day
Normal control	90±1.02**	92±1.08**	91±0.92**	93±0.87**
Diabetic control (Vehicle)	296±2.12	291±2.04	293±1.62	295±2.64
Alloxan+Glibenclamide (10 mg kg^{-1})	295±2.52**	211±2.74**	158±1.29**	149±1.96**
Alloxan+Methanolic extract of *Caesalpinia pulcherrima* (100 mg kg^{-1})	294±1.87**	278±2.08**	273±1.71**	263±2.07**
Alloxan+Methanolic extract of *Caesalpinia pulcherrima* (200 mg kg^{-1})	295±1.58**	255±2.96**	247±1.58**	242±3.17**
Alloxan+Methanolic extract of *Caesalpinia pulcherrima* (400 mg kg^{-1})	290±1.77**	242±2.45**	238±2.15**	233±2.61**

experimental diabetic animal models of human insulin-dependent diabetes mellitus. There is increasing evidence that alloxan caused diabetes by rapid depletion of a cells, by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. It leads to a reduction in insulin release there by a drastic reduction in plasma insulin concentration leading to stable hyperglycemic states. In this study significant hyperglycemia was achieved within 48 h after alloxan (150 mg kg^{-1} b.w. i.p.) injection. Alloxan induced diabetic rats with more than 200 mg dL^{-1} of blood glucose were considered to be diabetic and used for the study. It is now established that there is a gradual decrease in beta-cell function and mass that may occur in individuals at high risk of developing type II diabetes. To prevent the loss of beta-cell function and mass, beta cell stabilization or regeneration must occur. The renewal of β-cells in diabetes has been studied in several animal models. For example epicatechin has been shown to act by β-cell regeneration. Similarly *Vinca rosea* extracts also cause regeneration of β-cell in alloxan-induced diabetic rats[33,34].

The present study demonstrated substantial increases in blood glucose levels after alloxan injection of the rats. Etuk and Muhammed[35] and Adeyi *et al.*[36] confirmed this increase in glucose levels to the reactive oxygen species induced by alloxan; this, in combination with a simultaneous huge raise in cytosolic calcium concentrations led to quick damage of pancreatic islet cells and associated decrease in synthesis/release of insulin.

Diabetes is a multifaceted, chronic disorder forcing uninterrupted medical care with multifactorial risk-reduction strategies ahead of glycemic control. Continuing patient self administration education and hold up are dangerous to prevent acute complications and reducing the risk of long-standing complications. Important evidence exists that supports a range of intrusions to improve diabetes outcomes[37].

Several synthetic drugs have been industrialized for the treatment of diabetes. However, these drugs have limits in terms of efficacy and side effects. Therefore, there is much interest in discovering natural treatments without negative side effects in diabetic patients. Plant materials seem to be the solution[38].

The use of flavonoids or flavonoid full foods reduces the risk of diabetes mellitus. On the other hand, saponins contain the capacity to reduce increased plasma blood glucose, hence, making it helpful in managing diabetes mellitus. The hypoglycaemic result of saponin is through restoration of insulin response, improvement in insulin indicates, increase plasma insulin and induction of insulin release from the pancreas[39]. In the same way, the existence of triterpenes inhibit enzymes involved in glucose metabolism and prevent the increase of insulin resistance thus normalizing insulin levels[40]. Though, the preliminary phytochemical analysis of Pods of *Caesalpinia pulcherrima* confirms the presence of triterpenes[27].

CONCLUSION

From the present study, it was concluded that extracts of pods of *C. pulcherrima* are capable of exhibiting reduce in blood glucose level in normal and alloxan monohydrate-induced diabetic rats. It is very hard to give a statement on the mechanism of in detail, since the study was not designed accordingly.

SIGNIFICANCE STATEMENT

This study discovers the effects of *Caesalpinia pulcherrima* extracts on blood glucose in normal and alloxan monohydrate-induced diabetic rats that can be beneficial for further exponential study and isolation of pure compounds which may prove to be potential antidiabetic agents. This study will help the researcher to uncover the critical areas of many parts of the plant that many researchers were not able to explore. Thus a new hypothesis may be proposed on the effects of *Caesalpinia pulcherrima* extracts on blood glucose in normal and alloxan monohydrate-induced diabetic rats." submitted for publication to Journal of Biological Sciences. It

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