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Assessment of *In vivo* Antidiabetic Activity of *Adenanthera pavonina* (*Fabaceae*) Extract in Alloxan-induced Diabetic Rats

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Abstract

Objective: *Adenanthera pavonina* is a deciduous tree commonly used in traditional medicine to treat various ailments such as boils, epilepsy, blood disorders, seizures, inflammation, and rheumatism. However, the use of this plant extract lacks scientific validation as to its effectiveness. **Materials and Methods:** This study aimed to investigate the *in vivo* antidiabetic activity of the methanolic extract of *A. pavonina* leaves in alloxanized diabetic rats. The preliminary phytochemical screening was performed. The antidiabetic property was evaluated by oral administration of plant extract at doses of 200 and 400 mg/kg of body weight and by profiling blood glucose levels at 1, 7, 14, and 21 days using a glucometer. **Results:** The phytochemical screening reveals the presence of alkaloids, glycosides, phenolic compounds, tannins, saponins, proteins, amino acids, and steroids. The blood glucose level of 200 mg/kg and 400 mg/kg of plant extract on day 21 of the study was 188.41 ± 8.46 mg/dL and 182.01 ± 9.21 mg/dL, respectively, compared to the diabetic control 291.01 ± 10.25 mg/dL. **Conclusion:** The methanolic extract from the leaves of *A. pavonina* showed dose-dependent antidiabetic activity in alloxanized diabetic rats. The results suggest that the plant extract has significant ($P < 0.01$) antidiabetic activity.

Keywords: Antidiabetic activity, *Adenanthera pavonina*, Alloxan, *Fabaceae*, Medicinal plant

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Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, particularly the eyes, kidneys, nerves, heart, and blood vessels.[1] Diabetes mellitus is a chronic disorder whose prevalence is constantly increasing throughout the world. As a result of this trend, it is rapidly becoming an epidemic in some countries around the world and the number of affected people is expected to double over the next decade due to increasing population aging, increasing the already existing burden on people, especially in underdeveloped countries.[2] Over the past three decades, despite significant advances in diabetes treatment, outcomes for patients are still far from perfect. These treatments have some drawbacks, including drug resistance (reduced efficacy), side effects, and even toxicity. For example, sulfonylureas lose their effectiveness after 6 years of treatment in 44% of patients. It is also said that hypoglycemic drugs cannot control hyperlipidemia.[3] In spite of the presence of antidiabetic drugs on the pharmaceutical market, the treatment of diabetes with medicinal plants is usually successful. Medicinal plant components with negligible toxicity and no side effects are notable therapeutic options for the treatment of this disease worldwide.[4] Plants are the vital basis for medicinal uses and potential bioactive components for the development of new chemotherapeutic agents.[5] The plants have been used in the treatment of diabetes mellitus. A wide variety of plant-derived active ingredients representing numerous classes of chemical compounds has shown potential for use in the treatment of diabetes.[6] During the past decade, the use of complementary and alternative medicines (for example, herbal remedies and dietary supplements) for the treatment of chronic diseases such as diabetes has increased dramatically around the world. However, herbal medicines are complex mixtures of several bioactive components that can modulate the enzymes that metabolize drugs, particularly cytochrome P450, and interact with prescription drugs through pharmacokinetic mechanisms.[7] Diabetes is a severe metabolic disorder and there are several commercially available medications to alleviate the symptoms of diabetes. However, these over-the-counter medications are expensive and associated with several complications. Herbal medicines are gaining in importance as they are affordable and also show better therapeutic effects with fewer side effects.[8]

Adenanthera pavonina belongs to the *Fabaceae* family, commonly known as the red pearl tree. It is an important medicinal plant of the Indian subcontinent. Various parts of *A. pavonina* have been used in traditional medicine for the treatment of asthma, boils, diarrhea, gout, inflammation, rheumatism, tumors and ulcers, and as a tonic.[9] Several phytoconstituents have

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been reported in the leaves of this plant, including octacosanol, dulcitol, β-sitosterol glycosides, and stigmasterol.[10] A. pavonina constituted a series of phytochemicals, which describe their uses for various therapeutic purposes. Plant can be used to treat various health conditions and act as hepatoprotective, antihyperlipidemic, antinociceptive, antioxidant, antitumor, antimicrobial, nephrolithiasis inhibitor, and carcinogenesis.[11] A. pavonina has long been used in the treatment of many diseases as traditional medicine. Studies of plant showed that leaves of this plant possess analgesic, anti-emetic, antifungal, antibacterial, antidiarrheal, and anti-inflammatory activity,[12,13] therefore considering the reported pharmacological activities of *A. pavonina,* this study aimed to investigate the *in vivo* antidiabetic activity of the plant's leaves to provide scientific validation of its efficacy.

Materials And Methods

Study Site

This study was accomplished at the Department of pharmacology, T.V.E.S. Hon. Loksevak Madhukarrao Chaudhari College of Pharmacy, District-Jalgaon, Maharashtra-425503, India.

Collection and Authentication of Plants Materials

The leaves of the *A. pavonina* (*Fabaceae*) were collected from Jijamata Udyan, Byculla, Mumbai. The plant was authenticated by J. Jayanthi, Scientist "D" at botanical survey of India, Western regional center, Maharashtra. Plant sample was deposited with voucher specimen reference no. BSI/WRC/IDEN.CER./2017/579.

Preparation of the Plant Extract

The dried leaves were mechanically ground to a fine powder and sieved through a 40 mm mesh screen. The resulting powder was stored in airtight plastic bags. Five hundred grams of powdered plant sample were extracted in a Soxhlet extractor sequentially with petroleum ether, chloroform, and methanol. The extracts were decanted into a clean conical flask and filtered through Whatman filter paper into another conical flask. The extracts were dried using a rotary evaporator and stored in a container at 4°C and protected from light, however, only methanolic extract was selected and evaluated for *in vivo* antidiabetic activity.

Phytochemical Screening

A preliminary phytochemical screening of the methanolic extract of the leaves of *A. pavonina* was carried out to detect the presence of various phytoconstituents such as alkaloids, glycosides, phenolic compounds, tannins, saponins, proteins, amino acids, and steroids.[14-16]

Experimental Animals

Wistar rats of both sexes weighing between 180 and 250 g were used for this research. Swiss albino mice weighing between 20 and 30 g were used for acute oral toxicity study. Experimental animals individually housed in polypropylene cages, kept under standard conditions such as 12 h of light and 12 h of dark cycle, temperature of 25 ± 30°C, and relative humidity of 35–60%, and the protocols related to animal experimentation have been approved by the Institutional Animal Ethics Committee according to the CPCSEA guidelines at T.V.E.S. Hon. Loksevak Madhukarrao Chaudhari College of Pharmacy, District-Jalgaon, Maharashtra, India.

Acute Toxicity Test

The acute toxicity study was conducted according to the Organization for Economic Cooperation and Development-425 guidelines. On an overnight fasting, five Swiss albino mice were orally administered 2000 mg/kg of the leaf extract. One group was kept as a normal control and was administered vehicle alone. The animals were observed individually for symptoms of toxicity and mortality, if any, and then periodically for the next 24 h, and then every 24 h for any signs of acute toxicity over a period of 14 days.[17,18]

Induction of Diabetes

In rats, diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (2, 4, 5, 6-tetraoxypyrimidine; 5-6-dioxyuracil), 150 mg/kg of body weight was dissolved in normal saline. After 72 h of alloxan injection, animals with elevated blood glucose levels of 200 mg/dL were confirmed to be diabetic and used for the experiment.[19]

Antidiabetic Activity

The antidiabetic activity of the methanolic extract of the leaves was studied in alloxanized diabetic rats. The animals were randomly divided into five groups of six rats in each group. Group I consisted of normal rats orally administered with only vehicle (Tween 80, 3% v/v in normal saline); Group II consisted of alloxan-induced diabetic rats administered orally with vehicle; Group III consisting of diabetic rats administered glibenclamide (reference drug at 5 mg/kg of body weight); Group IV composed of experimental diabetic rats to which plant extract was administered at 200 mg/kg of body weight; and Group V formed by experimental diabetic rats to which plant extract was administered at 400 mg/kg of body weight. The blood glucose levels of the animals were estimated on days 1, 7, 14, and 21 using the glucometer.

Body Weight Determination

Body weights of all experimental rats were documented on treatment period days 1, 7, 14, and 21. An appropriately adjusted electronic balance was used for measuring body weight of the experimental animals.

Statistical Analysis

The results of statistical analysis were expressed as Mean ± S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used. Statistically, $P < 0.05$ was considered statistically significant. Extract treated groups were compared diabetic control group.

Results

The current study was done to evaluate the antidiabetic activity of methanolic extract of *A. pavonina* leaves in alloxan-induced diabetes Wistar rats. In the preparation of crude methanolic extract from the dried leaves, a yield 12.8% was obtained.

Phytochemical Screening

The results of preliminary phytochemical screening revealed the presence of phytoconstituents such as alkaloids, glycosides, carbohydrates, phenolic compounds, tannins, terpenoids, saponins, and sterols in *A. pavonina* leaves methanolic extract [Table 1].

Acute Toxicity Study

During the whole experimental period, no signs and symptoms of acute toxicity and mortality up to 2000 mg/kg body weight dose were observed. The food consumption was normal compared to vehicle treated mice. For further studies, the doses were fixed as 200 and 400 mg/kg body weight.

Effect of oral administration of methanolic leaf extracts of *A. pavonina* on blood glucose levels in alloxan-induced diabetic Wistar rats

A noticeable increase in fasting blood glucose level was obtained in alloxan-induced diabetic rats compared with the normal control group. According to one-way ANOVA analysis, there was significant difference among diabetic control and the group that received the standard drug. Result shows that significant drops in the blood glucose level in diabetic rats compared to diabetic control at 14 days. In addition, a similar result was detected at 21 days to that at 14 days [Table 2]. It was observed that the standard drug glibenclamide lowered the blood glucose level significantly, bringing it nearly back to normal, whereas leaves extract of both doses significantly ($P < 0.01$) decreased fasting blood glucose in the diabetic rats on the 7th, 14th, and 21st days as compared to initial 1st day [Figure 1].

Effect of the Methanolic Leaf Extracts of *A. pavonina* on Body Weights of Rats

At the end of experimental study, body weights of rats in normal control group (non-diabetic) were increased compared to their original body weights [Table 3]. Methanol extract of two doses (200 and 400 mg/kg body weight) treated groups rats did not significantly increased ($P > 0.05$) in body weight when compared to diabetic rats in control group [Figure 2].

Discussion

The present study discusses the antidiabetic effect of *A. pavonina* leaf extract in alloxan-induced diabetic rats. The antidiabetic effect of the leaf extract could be attributed to the presence of phytoconstituents such as alkaloids, glycosides, carbohydrates, phenolic compounds, tannins, terpenoids, saponins, and sterols that have been associated with possible activity [Table 1]. Acute toxicity studies revealed the non-toxic nature of the extract. No lethal or toxic reactions were observed with the selected dose until the end of the study period. Alloxan causes a massive reduction in insulin release by destroying the lymphocytes of the B cells of islet of Langerhans, which induces hyperglycemia.[20] Treatment with alloxan causes a sudden increase in insulin secretion in the presence or absence of glucose and this insulin release occurs for a short period of time followed by complete suppression of the islet response to glucose even when they have used high concentrations of glucose.[21] Alloxan monohydrate-induced diabetic rats showed a persistent increase in blood glucose level after 7 days with the characteristics of diabetes mellitus.[22] Treatment of diabetic rats with *A. pavonina* leaf extract at 200 mg/kg and 400 mg/kg for 21 days caused a significant reduction in fasting glucose in a dose-dependent manner compared to the diabetic control group. The extract-treated diabetic rats also showed an improvement in their body weight.

The improvement of liver function and the consequent increase in the absorption of glucose into the blood and its use, it may be mechanism of action of the extract. Another possible mechanism includes cell stimulation and subsequent release of insulin and activation of insulin receptors.[23] Estimating insulin level and insulin receptor can provide additional information in the mechanism of the antidiabetic activity exhibited by the extract.

In light of the results, the study indicates that the methanolic extracts of the leaves of *A. pavonina* have a good antidiabetic

Table 1: Phytochemical screening of *Adenanthera pavonina* leaves

Phytoconstituents	*Observation*
Alkaloids	+
Glycosides	+
Saponins	+
Tannins	+
Lipids	-
Sterols	+
Terpenoids	+

Presence of phytoconstituents represented by (+) sign, absence of phytoconstituents represented by (-) sign

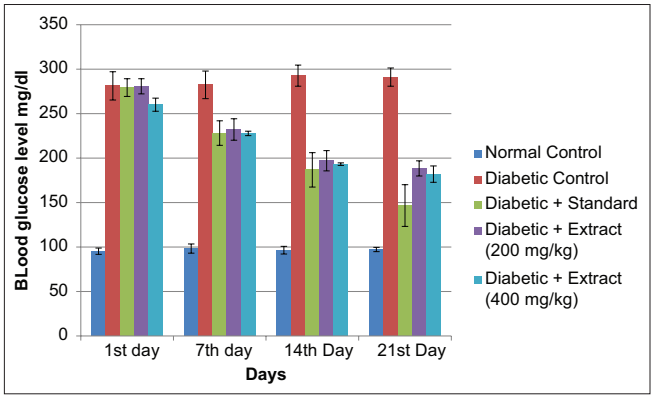

Figure 1: Effect of *Adenanthera pavonina leaves* methanolic extract on blood glucose level of rats

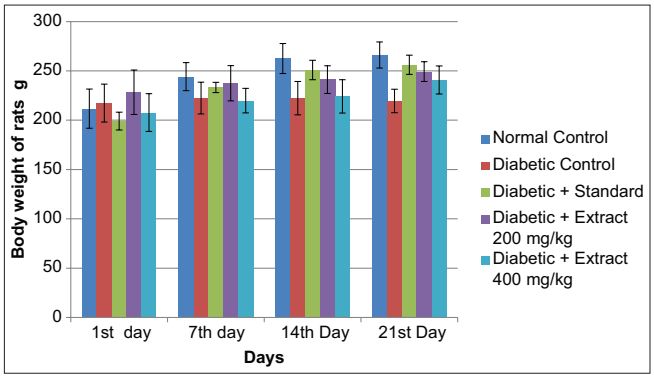

Figure 2: Effect of *Adenanthera pavonina* leaves methanolic extract on body weight of rats

Table 2: Effect of *Adenanthera pavonina* leaves methanolic extract on blood glucose level of rats (mg/dL)

Groups	*1st day*	*7th day*	*14th day*	*21st day*
Normal control	95.33±3.63	98.33±5.16	96.5±4.29	97.21±2.38
Diabetic control	281.17±15.814	282.33±15.54	292.67±11.935	291.01±10.25
Diabetic+Standard	279.17±9.96	228.17±13.81**	186.83±19.321**	146.7±23.42**
Diabetic+Extract (200 mg/kg)	280.63±8.51	232.09±12.07**	197.04±11.38**	188.41±8.46**
Diabetic+Extract (400 mg/kg)	260±7.24	227.75±2.57**	193.15±1.43**	182.01±9.21**

Values are Mean±S.E.M, *n*=6, ***P*<0.01 respective diabetic control (one-way ANOVA followed by Dunnett's test)

Table 3: Effect of *Adenanthera pavonina* leaves methanolic extract on body weights of rats (g)

Groups	*1st day*	*7th day*	*14th day*	*21st day*
Normal control	211.67±19.861	244.17±14.27	262.5±15.259	266.10±13.30
Diabetic control	217.33±19.237	222.5±16.099	222.33±16.848	219.46±11.854
Diabetic+Standard	199.17±9.035	233.16±5.115^{ns}	250.83±9.867^{ns}	256.13±9.778 ^{ns}
Diabetic+Extract 200 mg/kg	228.35±22.47	237.49±17.87^{ns}	241.18±14.04^{ns}	249.27±10.01^{ns}
Diabetic+Extract 400 mg/kg	207.78±19.08	219.84±12.37^{ns}	224.17±16.88^{ns}	240.74±14.22 ^{ns}

Values are Mean±S.E.M, *n*=6, ^{ns}*P*>0.05 respective diabetic control (one-way ANOVA followed by Dunnett's test)

activity. The extract can be used in conjunction with other established antidiabetic medications or herbal formulations for more effective results.

Conclusion

This study demonstrated that *A. pavonina* leaves possess antidiabetic effects in alloxan-induced diabetic rats, scientifically validating its continued use in the management of diabetes mellitus. The antidiabetic activity was due to the cumulative effect of the phytochemicals present in the plant extract, including alkaloids, tannins, saponins, and total phenols. However, further research should be done focusing on the isolation of the bioactive molecules responsible for the antidiabetic effect of this plant through bioassay-guided fractionation.

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