

ORIGINAL ARTICLE

# Hypoglycemic Activity of Extracts from *Elytraria Acaulis L*. Leaves in Alloxan-Induced Diabetic Rats

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# ABSTRACT

The prevalence of diabetes mellitus is increasing with ageing of the population and life style changes associated with rapid urbanization and westernization. The aim of this study was to producing an inventory of *Elytraria acaulis* plant used by traditional healers in Adilabad district of Andhra Pradesh to treat diabetes. The stem, leaves, root and flowers of *E. acaulis* was collected from the local areas of Adilabad district, Andhra Pradesh, India. The powdered plant parts were successfully extracted with boiling water using soxhlet extractor. A preliminary toxicity study of *E. acaulis* crude extracts was done using seven main groups of male Wister albino rats. The antihyperglycemic activity of the crude aqueous extracts of *E. acaulis* different parts were studied in alloxan-induced diabetic rats, after oral administration at a dose of 250 mg/kg body weight for a period of 28 days. The toxicity study results showed that the medium lethal dose (LD<sub>50</sub>) of the extracts is higher than 1g/kg body weight. There is no significant level of reduction in fasting blood glucose level was noticed for the aqueous extracts of root and stem of *E. acaulison*. On chronic administration, the effect of *E. acaulis* flower and leaf causes a fall in fasting blood sugar of rats. A significant increase in the levels of cholesterol, and triglycerides were observed in diabetic rats when compared to normal control groups.

Keywords: Elytraria acaulis, Diabetes mellitus, Adilabad, Alloxan-induced.

#### INTRODUCTION

Diabetes mellitus is a disease results from abnormality of carbohydrate metabolism and characterized by absolute or relative deficiencies in insulin secretion or receptor insensitivity to endogenous insulin, resulting in hyperglycemia (ELHilaly et al., 2007). Diabetes mellitus is a complex disorder that characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action both causing by impaired metabolism of glucose, lipids and protein (Scheen, 1997). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs (Lyra et al, 2006).

Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes

and the related complications continued to be a

medical major problem. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically antidiabetic as and antihyperlipidemic remedies (Mitra et al, 1996). Antihyperglycemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes.

More than 400 plant species having hypoglycemic activity have been available in literature (Oliver-Bever, 1986 and Raj, 1995)), however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., that are frequently implicated as having antidiabetic effect (Loew and Kaszkin, 2002).

The Common name of Elytraria acaulis (Acanthaceae) is Asian Scaly stem. It is a stem less perennial herb with one to several unbranched flowering stems, up to 30 cm. This plant is often found on often on rocky or sandy soils. As per the Ethnobotanical literature on traditional phytotherapy of Indian medicinal the species *Elytraria acaulis* plants. is consistently used by the tribal communities for the treatment of diabetes as well as in modern medicine. There is no any scientific evidence is available of this plant to treat diabetes. Therefore this work has been taken with the aim of producing an inventory of this plant used by traditional healers in Adilabad district of Andhra Pradesh to treat diabetes.

#### MATERIAL AND METHODS

#### **Plant Material**

The stem, leaves, root and flowers of *Elytraria acaulis* was collected from the local areas of Adilabad district, Andhra Pradesh, India, identified and authenticated from botanist Dr. A.V.S.S. Raju, Department of Botany, Kakatiya University, Warangal and the voucher specimens of *Elytraria acaulis* is preserved in the Department of Zoology. The different parts of plant material were placed in brown paper bags and dried in the drying room and ground into fine powder for extraction.

#### Figure-1: Pictures of Elytraria acaulis





#### **Preparation of extracts**

The collected plant parts of stem, leaves, root and flowers were cleaned and washed well with water. Then 50 g of selected plant parts were dried under shade at  $25^{0}$ C for 5 days in the absence of sunlight and grounded well to fine powder. The powdered plant parts (nearly 30 g) were successfully extracted with boiling water using soxhlet extractor are then cooled and filtered using Whatmann No 1 filter paper. The filtrate was centrifuged at 10,000 rpm at room temperature ( $25^{0}$ C) and the sediment was discarded. The supernatant was concentrated up to 100 mL on rotavapour under reduced pressure. The concentrated crude extract was lyophilized into powder (5 g) and used for the study.

# **Experimental animals**

The Wister strains of male albino rats weighing between 100-150 g were obtained for the present study, from National Institution of Nutrition, Hyderabad. The animals were housed in larger spacious cages and they were fed with commercial pelleted rat chow marketed by Hindustan Lever Ltd. Bangalore India under the trade name Gold Mohur Rat Feed and had free access to water ad labium. The animals were well acclimatized to standard environmental conditions of temperature (22°C+5°C) and humidity (55+5%) and 12 h light dark cycles throughout the experimental period. The animals used in the present study were approved Ethical by the institution Committee (134/ZOO/KU/2012).

## Induction of experimental diabetes

The overnight fasted rats were infected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body wt (Katsumala *et al*, 1999). The control rats received the same amount of saline solution. After one hour of alloxan administration, animals were given fed *ad libitum* and 1 mL of (100 mg/mL) glucose i.e., combats ensuring severe hyperglycemia. After 72 h of the alloxan injection, the animals were tested fro evidence of diabetes by estimating their blood glucose level by using glucose estimation kit. The blood glucose level more than 150 mg/100 mL of blood was criteria.

#### **Experimental design**

The rats were segregated into 7 groups with minimum of 8 rats in each group.

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Group I : Normal control rats; Group II: Diabetic control rats; Group III: Diabetic rats treated with stem extract of EA (Elytraria acaulis); Group IV: Diabetic rats treated with leaf extract of EA]; Group V : Diabetic rats treated with root extract of EA; Group VI: Diabetic rats treated with flower extract of EA

Group VII: Diabetic rats administered with Tolbutamide (150 mg/kg bw) in aqueous solution Valand blogdygluaosed davel SEMmetasixed intal, in Pach group orally for 28 days.

#### **Treatment Protocol**

Test extracts (250 mg/kg b.w.), standard drug Tolbutamide (150 mg/kg b.w) and control (2 mL saline) were administered orally, every 24 h for a period of 28 days. Animals described as festered were deprived of food for at least 12 h but allowed free access to drinking water. The blood samples were obtained through the tail vein puncturing with hypodermic needy under light ether anesthesia. 0.2 mL of blood was withdrawn at interval of initial, 1, 3 and 5<sup>th</sup> h of administration of single dose (for acute study) and at the end of 7, 14, 21 and 28<sup>th</sup> days (prolonged study). Blood was collected and centrifuged at 3000 rpm for 15 min to separate plasma.

#### Acute toxicity test

Seven main groups of male Wister albino rats were selected to study the acute toxicity of all plant extracts under investigation. All groups received one oral dose of 100, 250, 500, 650, 800, 950 and 1100 mg of plant extract/kg body weight. Animals were kept under close observation for 24 hours after administering the extract, and then they were observed daily for three days for any change in general behaviour and/or other physical activities. After 24 hours,

there were no died animals; representing the safety action of all extracts.

#### **Biochemical analysis**

The blood glucose was measured in all the groups by using glucose enzyme reagent system manufactured by Span Diagnostic Private Ltd, Surat, India. The oral glucose tolerance test was performed on overnight fasted normal, diabetic and treated rats on 21<sup>st</sup> day of the treatment. Glucose (2 g/kg body weight) was given orally

60, 90 and 120 min after administration of glucose.

#### **Statistical analysis**

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean  $\pm$  S.D. The significant differences among values were analyzed using analysis of variance (one-way ANOVA) in latest computer software programme.

#### **RESULTS AND DISCUSSION**

#### **Body Weight**

Table-1 shows body weight was decreased significantly in allexon-induced diabetic rats in respect to control animals. Treatment of extract of Elytraria acaulis to diabetic rat resulted, a significant recovery in this parameter. The recovery in the level of this parameter was significant recovered in composite extract treated diabetic group when the comparison was made with the individual extract treated diabetic group. A significant difference was maintained in the level of this parameter in Tolbutamide treated group in respect to composite extract treated group.

Table-1: Effect of extracts of Elytraria acaulis (EA) Body weight in Diabetic control and allexon-Induced Diabetic Rats.

Variables	Normal Control (Group I)	Diabetic Control (Group II)	Stem extract of E.A (Group III)	Leaf extract of E.A (Group IV)	Root extract of E.A (Group V)	Flower extract of E.A (Group VI)	Tolbutamide Treated (Group VII)
Body weight(g) Initial	262.40 ± 17.60	269.50 ± 13.90	262.50 <u>+</u> 14.20	263.50 <u>+</u> 15.50	261.50 <u>+</u> 14.50	262.40 <u>+</u> 14.20	264.6 <u>+</u> 23.30
Body weight(g) final	271.80 ± 13.55	$182.00 \pm 10.70$	$\begin{array}{r} 148.50 \pm \\ 4.80 \end{array}$	150.90 <u>+</u> 5.10	152.50 <u>+</u> 5.90	149.40 <u>+</u> 5.50	149.60 <u>+</u> 15.90



Figure-2: Effect of *Elytraria acaulis* on blood glucose level of alloxan induced diabetic rats after single dose

## Acute Toxicity Test

Acute toxicity studies conducted revealed that the administration of graded doses of stem, leaf, root and flower crude extracts (up to a dose of 1100 mg/kg) of *Elytraria acaulison* did not produce significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and appearance of the animals. No death was observed up to the dose of 1 g/kg body weight. The mice were physically active. These effects were observed during the experimental period (72 hrs). The result showed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD<sub>50</sub>) could be greater than 1 g/kg body weight in mice.

# Effect Of *Elytraria acaulis* On Blood Glucose Level

Glucose is a substance and an indispensable which supplier, supports energy cellular function. Glucose measurements are used in the diagnosis and monitoring carbohydrate metabolism disorders including diabetes mellitus, neonatal hypoglycemia, idiopathic hypoglycemia and pancreatic islet carcinoma (Virella Lopes and Virella, 2003). In recent years, various plant extracts havbe been claimed to be useful for the treatment of diabetes mellitus

(Shukla et al, 2000). Over 150 plant extracts and some of the active principle including flavonoids are known to be used for the treatment of diabetes (Bailey, 1989; Choi et al, 1991).

Alloxan, a beta cytotoxin, induces chemical diabetes (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic-cell, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues (Shukla et al, 2000). The mean fasting glucose levels for group I to group VII are indicated in Figure-2 (for acute study) and Figure-3 (for prolonged study).

Results in Figure-2 and 3 shows that there was an elevation in blood glucose levels in alloxan treated diabetic rats when compared with normal rats. The intraperitoneal injection of alloxan in Wister rats produced hyperglycemial impaired glucose tolerance and insulin resistance. Among the administration of the extracts of various plant parts of *E. acaulison*, only the flower and leaf extracts and tolbutamide tends to bring the fasting blood glucose level towards the normal in the acute study. There is no significant level of reduction in fasting blood glucose level was noticed for the aqueous extracts of root and stem of *E. acaulis*.



Figure-3: Effect of *Elytraria acaulis* on blood glucose level of alloxan induced diabetic rats after prolonged treatment

On chronic administration, (Table 2) the effect of *E. acaulison* flower Et (133.77+3.167) and leaf Et (134.33+2.280) causes a fall in fasting blood sugar of rats. The fall is evident even in the 1<sup>st</sup> week and goes on progressively increasing till at the end of 4 weeks and the fall in the fasting blood sugar was nearly equal to that of reference drug Tolbutamide (126.60+2.555). The anti-diabetic activity of root and stem is not significant in prolonged study also. These findings clearly established that the anti-diabetic efficacy of the flower and leaf extract of *E. acaulison* are almost equal and both exhibited more potent antidiabetic activity by reducing the blood glucose level significantly than all other root and stem extracts.

In this study, we have observed that aqueous extracts of Flower and leaves of *Elytraria acaulison* decreases blood glucose in alloxan diabetic rats. The reason may be flowers and leaves contain more constituents such as alkaloids, steroids and tannin's. Hence the more potent antidiabetic activity of *Elytraria acaulison* leaves and flower extracts may be due to nature of more alkaloids, steroils or tannins present in them. Like the plant extract, Tolbutamide also produced significant reduction in blood glucose levels of alloxan diabetic rats.

In this study, we have also observed an increase in the concentration of total cholesterol, triglycerides, in alloxan untreated diabetic rats. Hyperlipidemia is a recognized consequence of diabetes mellitus (Pushparaj and Tan, 2000; Pepato *et al.*, 2003; Sharma *et al.*, 2003). Diabetes induced hyperlipidemia is attributable to excess mobilization of fat from the adipose due to the under utilization of glucose (Krishnakumar *et al.*, 2000). The ability of aqueous extracts of stem, leaves, root and flowers of *E.acaulison* to reduce the levels of plasma lipids in diabetic rats has never been studied before.

#### CONCLUSION

This study concludes that, the leaves and flowers of Elvtraria acaulison extracts has cardio protective potential along with antidiabetic effects possess beneficial and effect on the hyperglycemia associated with hyperlipidemia. In single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD<sub>50</sub>) could be greater than 1 g/kg body weight in mice. Body weight was decreased significantly in allexon-induced diabetic rats in respect to control animals. On chronic administration, the effect of E. acaulison flower and leaf extracts causes a fall in fasting blood sugar of rats.

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DOI: https://dx.doi.org/10.5281/zenodo.7180885 Received: 10 January 2013; Accepted; 26 February 2013; Available online : 4 March 2013