

Toxicological evaluation and oral glucose tolerance test of ethanolic leaf extract of *Barleria cristata* L. in wistar albino rats

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ABSTRACT

Background: To evaluate the acute toxicity study and effective dose determination of ethanolic leaf extract of *Barleria cristata* L.

Methods: Toxicological evaluation and effective dose determination of ethanolic leaf extract of *Barleria cristata* were performed in wistar albino rats. 250, 500, 1000 and 2,000 mg/kg of body weight of ethanolic leaf extract of *Barleria cristata* (EtBc) were administered orally as a single dose to rats. Rats were observed periodically for symptoms of toxicity and death within 24 hours and then daily for the next 14 days. So the rats were observed for another 14 days and then sacrificed to collect serum and organs for the analysis of biochemical parameters. After this study, rats were induced with diabetes by a single intra peritoneal injection of 45 mg/kg bodyweight of streptozotocin. Ethanolic leaf extract of *Barleria cristata* was orally administered to diabetic rats at 200, 400 and 600mg/kg doses for 7 days through oral glucose tolerance test (OGTT). Glycemic index was demonstrated the variable doses of ethanolic leaf extract in normal and diabetic rats during OGTT studies.

Results: In acute toxicity study, the results were showed that the administration of the ethanolic leaf extract of *Barleria cristata* (EtBc) at all given doses (up to 2000 mg kg) did not produce any sign of acute toxicity or instant death in rats tested during the period of observation. From OGTT study, 400mg/kg dosage of EtBc exhibited notable blood glucose lowering effect at 90 min than the other doses and this was similar to that of standard drug glibenclamide treated rats. This dosage was showed the highest percentage of glycemic index in both normal and diabetic rats.

Conclusion: EtBc was revealed the non-toxic nature used for acute toxicity studies and among various doses of this extract, 400 mg/kg brought an effective hypoglycemic activity in wistar albino rats.

Keywords: Diabetes mellitus, Medicinal plant, Hyperglycemia

INTRODUCTION

The oral glucose tolerance test (OGTT) is a widely used procedure in the diagnosis of diabetes and intermediate stages of hyperglycemia practical attempt to simplify and facilitate the diagnosis of diabetes.¹ Diabetes mellitus (DM) consists of a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, and proteins, and an increased risk of complications from vascular disease.² Apart from currently available therapeutic options for diabetes like oral hypoglycemic agents and insulin, which have limitations of their own, many herbal medicines have been recommended for the treatment of diabetes.³

Evaluation of plant products to treat diabetes mellitus is of growing interest as they contain many bioactive

substances with therapeutic potential. In recent years, several authors evaluated and identified the antidiabetic potential of traditionally used Indian medicinal plants using experimental animals. A variety of ingredients present in medicinal plants are thought to act on a variety of targets by various modes and mechanisms. They have the potential to impart therapeutic effect in complicated disorders like diabetes and its complication.⁴

Barleria cristata L. is known as Philippine violet belongs to the family of Acanthaceae. *Barleria* genus has 300 species of herbs with a variety of medicinal activities and uses. *Barleria cristata* has been used as a traditional herbal remedy in Thailand and it allegedly acts as a tonic, diuretic and blood purifier.⁵ This plant has also been reported as traditional medicine which used for many countries. Based on the above knowledge the present

study was designed to find the best extract of *Barleria cristata* for antihyperglycemic studies through the pilot study and then the selected extract was subjected to toxicological studies to find the safety of the drug. After then the effective dose of the selected extract was determined by performing the oral glucose tolerance test (OGTT).

METHODS

Plant materials

The leaves of *Barleria cristata* used for the investigation were obtained from Coimbatore district, Tamilnadu, India. The plant was authenticated in Botanical Survey of India, TNAU Campus, Coimbatore and the voucher specimen No. is BSI/SRC/5/23/2011-12/Tech.-n62. The leaves of *Barleria cristata* air dried at 25°C for 10 days in the absence of sunlight and powdered well using a mixer and stored in an air tight container. The plant residue was extracted with 250ml ethanol by using a soxhlet apparatus for 8–10 hours.

Animals

Wistar albino rats of either sex weighing about 150–180g were procured from the animal house of Karpagam University, Coimbatore, India. The animals were under standard conditions of 12 hr light and 12 hr dark cycle, with temperature of $24 \pm 2^\circ\text{C}$, relative humidity of $50 \pm 10\%$ and fed with rodent diet and water *ad libitum*. The study was approved by Institutional Animal Ethical Committee constituted for the purpose of CPCSEA, Government of India.

Experimental design for acute toxicity study

250, 500, 1000 and 2000 mg/kg body weight of EtBc was administrated orally as single dose to various groups of rats (table 1) and each group comprises a total of six rats. The suspensions were prepared in water and given at a dose of 1.0 ml to the experimental animals and the experimental design is given in table 1.

Table 1: Toxicology evaluation of experimental design.

Groups	Experimental design
Group I	Control, normal healthy rats
Group II	Rats were treated with 250 mg/kg dosage of EtBc
Group III	Rats were treated with 500 mg/kg dosage of EtBc
Group IV	Rats were treated with 1000 mg/kg dosage of EtBc
Group V	Rats were treated with 2000mg/kg dosage of EtBc

After the administration of the extract, the animals were observed individually for a period of 24 hrs for the signs of toxicity or mortality. In acute toxicity study even though the rats appeared healthy and normal, it is mandatory to monitor the rats daily for atleast 1 to 2 weeks to find out the delayed toxicity. So the rats were observed for another 14 days and then sacrificed to collect serum and organs for the analysis of biochemical parameters includes blood glucose⁶, body weight, alanine transaminase (ALT), aspartate transaminase (AST)⁷, urea⁸, uric acid⁹ and creatinine.¹⁰

Induction of diabetes

Rats were fasted overnight before inducing diabetes with streptozotocin. The rats were given an intraperitoneal injection of streptozotocin (45 mg/kg) freshly prepared in 0.1M sodium citrate buffer. The diabetic state was confirmed 48 hrs after streptozotocin injection.

Experimental design for Oral Glucose Tolerance Test (OGTT)

The experimental rats, who had fasted overnight, were randomly divided into nine groups of four rats of each. Control group rats were received 1.0 ml of physiological saline.

Table 2: Effective dose determination of experimental design.

Groups	Experimental design
Group I	Control, normal healthy rats
Group II	Diabetic control
Group III	Diabetes + 200 mg/kg bw EtBc for a period of 7 days
Group IV	Diabetic + 400 mg/kg bw EtBc for a period of 7 days
Group V	Diabetic + 600 mg/kg bw EtBc for a period of 7 days
Group VI	Diabetic + 1.25 mg/kg bw Glibenclamide drug for a period of 7 days
Group VII	200 mg/kg bw EtBc alone for a period of 7 days
Group VIII	400 mg/kg bw EtBc alone for a period of 7 days
Group IX	600 mg/kg bw EtBc alone for a period of 7 days

After the treatment period, on 7th day the rats were fasted overnight with free access to water. Fasting blood sample was drawn from the tail and blood glucose level was measured by using glucometer. Then all the rats were loaded with 2 g/kg glucose solution and then orally treated according to their respective grouping. Six more

blood samples were collected at 30, 60, 90, 120, 150 and 180 minutes of EtBc treatment.

Glycemic index for OGTT

From the OGTT study, initial and final blood glucose levels were noted and glycemic index was calculated by the formula

$$\% \text{ Glycemic index} = \frac{\text{Initial blood glucose} - \text{final blood glucose}}{\text{Final blood glucose}} \times 100$$

RESULTS

Acute toxicity studies of the ethanolic leaf extract of *Barleria cristata* (EtBc)

In the acute toxicity study, 250, 500, 1000 and 2,000 mg/kg of body weight (bw) of ethanolic leaf extract of *Barleria cristata* was administered orally as a single dose to Wistar albino rats. They were observed periodically for symptoms of toxicity and death within 24 hours and then monitored daily for the next 14 days.

The effect of *Barleria cristata* leaf extract on the body weight of rats was shown in the figure 1. After 14 days the body weights were measured. No significant changes

were observed in the body weight of the EtBc treated rats. Biochemical studies such as glucose, hepatic markers include AST and ALT and renal markers include urea, uric acid and creatinine were assessed. The effect of EtBc on these markers of control and experimental rats were shown in the Table 3.

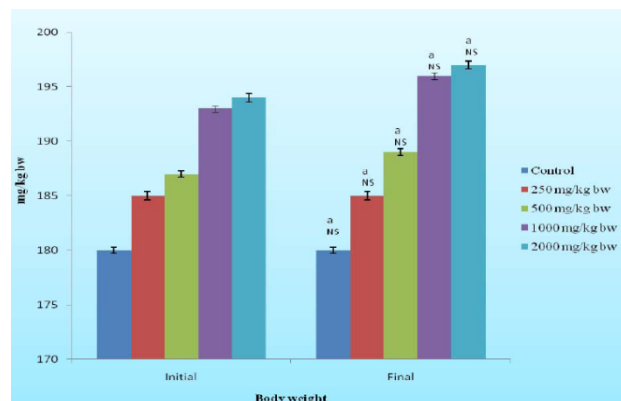


Figure 1: Effect of EtBc on body weight of experimental rats.

a: Final body weight compared to initial body weight

NS: Non-significant at 95% level (P<0.05%)

Table 3: Effect of EtBc on the biochemical parameters in the serum of experimental rats.

Groups	Doses (mg/kg bw)	Glucose (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	ALT (U/L)	AST (U/L)
I	Control	89.05±2.21 ^a	21.08±1.62 ^a	2.3±0.59 ^a	0.90±0.02 ^a	18.07±1.0 ^a	20.12±1.0 ^a
II	250	89.10±2.05 ^a	21.17±1.95 ^a	2.4±0.87 ^a	0.94±0.04 ^a	18.21±1.5 ^a	20.58±1.5 ^a
III	500	89.25±2.08 ^a	21.27±2.15 ^a	2.5±0.45 ^a	0.95±0.03 ^a	18.55±1.6 ^a	20.71±1.2 ^a
IV	1000	89.30±2.25 ^a	21.45±2.53 ^a	2.7±0.91 ^a	0.97±0.02 ^a	18.72±1.2 ^a	20.84±1.2 ^a
V	2000	89.52±2.25 ^a	21.82±2.09 ^a	2.9±0.92 ^a	0.99±0.04 ^a	18.94±1.7 ^a	20.89±1.4 ^a

Values are expressed as mean ± SD for six animals in each group.

Values not sharing common superscript letters ^(a) differ significantly at p<0.05 (DMRT).

The results in this study showed that the administration of the ethanolic leaf extract of *Barleria cristata* at all given doses (up to 2000 mg/kg) did not produce any sign of acute toxicity or instant death in rats tested during the period of observation.

Effect of EtBc on OGTT of normal and diabetic rat

After the administration of EtBc for 7 days with different dose levels (200, 400 and 600 mg/kg), the OGTT test was performed in overnight fasted diabetic and normal rats and the result was shown in figure 2. The blood samples were analysed for glucose at fasting, 30, 60, 90, 120 and

180 minutes. From this study, 400mg/kg dosage of EtBc exhibited notable blood glucose lowering effect at 90 min than the other doses and this was similar to that of standard drug glibenclamide treated rats. The significant reduction of blood sugar within 90 min manifests the hypoglycemic potential of EtBc in rat models.

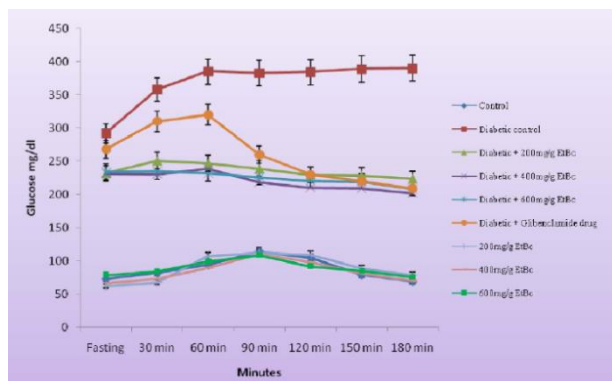


Figure 2: Effect of various doses of EtBc on GTT in normal and diabetic rats.

Effect of glyceimic index on OGTT

Figure 3 demonstrates the glyceimic index for variable doses of ethanolic leaf extract in normal and diabetic rats during GTT studies. Glyceimic index range of 1.96, 4.5 and 3.14% was observed after 3 hrs of glucose administration with the doses of 200, 400 and 600mg/kg respectively in treated diabetic rats. 1.47, 5.06 and 3.94% was observed with the doses of 200, 400 and 600mg/kg respectively in normal rats. From this result, it was confirmed that 400mg/kg showed the highest percentage of glyceimic index in both normal and diabetic rats.

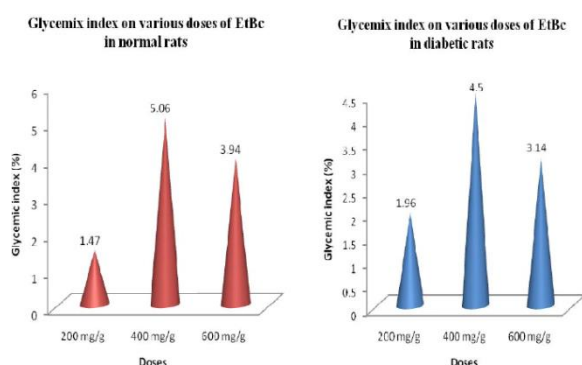


Figure 3: Effect of EtBc on glyceimic index of normal and diabetic rats.

DISCUSSION

The type of toxicity tests which are routinely performed by pharmaceutical manufacturers for a new drug involves acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD₅₀ (the dose which has proved to be lethal, causing death to 50% of the tested group of animals). Determination of acute oral toxicity is usually an

initial screening step in the assessment and evaluation of the toxic characteristics of all compounds.¹¹

From the results, it is clear that the extract at different doses were not toxic which was proved by unalteration of body weight¹² and did not produce any toxic sign of experimental rats. Therefore, the expected high LD₅₀ (>2000 mg kg/body weight) of the ethanolic leaf extract of *Barleria cristata*, is an indication that the extract could be considered relatively safe especially when administered orally where absorption may not be complete due to inherent factors limiting absorption in the gastro intestinal tract. In addition, in all the tested dose the ethanolic extract proved that it is devoid of renal and hepatic toxicity which was evidence from the unalteration of renal and hepatic markers. Although, obtaining a lethal dose in animals may not predict the human lethal dose of a drug or acute poisoning overdose.¹³ However, it is usually used to provide a guideline for selecting doses for acute dosage for future clinical relevance.

The oral glucose tolerance test (OGTT) is a widely used procedure in the diagnosis of diabetes and intermediate stages of hyperglycemia.¹⁴ In diabetic condition OGTT determine the malabsorption may be associated with insulin secretion and insulin resistance. It measures the body's ability to use a type of sugar, called glucose that is the body's main source of energy. GTT, a test of immense value and sentiment, in favor of using fasting plasma glucose concentration alone was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes. This study was showed an appreciable improvement in glucose tolerance which could be attributed to the insulin mimetic activity of *Barleria cristata* by restoring the insulin response.¹⁵ Hence, it was confirmed that 400mg/kg showed the highest percentage of glyceimic index in both normal and diabetic rats.

High glyceimic index diet produced persistently high level of insulin secretion from the pancreas, which ultimately resulted in post-receptor insensitivity to the released insulin. Ethanolic leaf extract of *Barleria cristata* at a dose of 400 mg/kg brought a very good glyceimic control when compared to other doses. Since the highest glyceimic index was associated with the dose of 400 mg/kg dose, this dose was used for further studies.

CONCLUSION

Ethanolic leaf extract of *Barleria cristata* was used for acute toxicity studies which revealed the non-toxic nature of the ethanolic leaf extract, since there were no deaths or lethal reactions up to a dose of 2000 mg/kg body weight in rats. Among the various doses (200, 400, 600mg/kg) of EtBc on OGTT in normal and diabetic rats, 400 mg/kg brought an effective hypoglycemic effect when compared to other doses. Therefore, this effective dosage, 400mg/kg of ethanolic leaf extract of *Barleria cristata*

was used for further antidiabetic studies in Wistar albino rats.

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Ethical approval: The study was approved by the Institutional Animal Ethical Committee

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