



## HYPOGLYCAEMIC ACTIVITY OF ETHANOLIC EXTRACT OF *LEPIDAGATHIS CRISTATA* WILLD IN ALLOXAN INDUCED DIABETIC RATS.

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### ABSTRACT

Type 2 Diabetes mellitus (DM) is the most common form of disease globally with rapidly developing countries being at the forefront of this epidemic. The synthetic drugs used for treatment are associated with several adverse effects and are expensive. The current focus has been shifted to treat DM and its complications through plant derived drugs due to their high efficacy and safety. Aim: In our study, the main objective is to find out the hypoglycaemic activity of ethanolic extract of leaves of *Lepidagathis cristata* Willd in alloxan induced diabetic rats and its comparison with the standard drug Glibenclamide (5mg/kg). Methodology: Wistar rats weighing 200-250gms were selected and diabetes was induced by injecting alloxan monohydrate (120mg/kg bodyweight) intra-peritoneally. Animals were divided into six groups, I group was kept as a normal, II group as a control group, III group was treated with standard drug Glibenclamide (5mg/kg). Remaining three groups were treated with different doses of (100,200 and 400mg/kg body weight) of ethanolic extract of leaves of *Lepidagathis cristata* for a period of 3weeks. Results were analyzed by estimating the fasting blood glucose levels. The effect of EELC leaf extract on blood glucose, serum enzymes SGOT, SGPT, ALP and TC, TG, LDL and HDL were measured on days 7, 14 and 21. Results: An ethanolic extract of *Lepidagathis cristata* showed a significant fall in fasting blood glucose at all the doses. But, at the dose of 400mg/kg showed activity on par with standard Glibenclamide.

**Keywords:** Diabetes, *Lepidagathis cristata*, Hypoglycaemia, Ethanolic extract, Alloxan. Glibenclamide.

### 1. INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder characterized by metabolic derangements of carbohydrates, fat and proteins, there by develops complications such as nephropathy, retinopathy, neuropathy and cardiomyopathy over the period of time. India expected to become the Diabetic capital of the world in the year 2025, during which maximum numbers of patients are seen<sup>1,2</sup>. Traditional medicines derived mainly from plants play major role in the management of diabetes mellitus<sup>3</sup>. In recent past, many medicinal plants possessing experimental and Clinical anti-diabetic activity that has been used in traditional

systems of medicine<sup>4</sup>. The drugs associated with DM like hypoglycemic agents such as biguanides and sulphonyl ureas are associated with several side effects and sometimes they are found to be ineffective in chronic diabetic patients and are expensive<sup>5</sup>. Ayurvedic literatures like Charaka Samhita and Sushruta Samhita, have reported the use of the grains for the management of diabetes mellitus<sup>6</sup>. Among adults, it is one of the leading causes of death also cases of legal blindness and lower extremities amputation<sup>7,8</sup>. In other hand, many synthetic hypoglycemic agents were introduced for maintenance of type 2 diabetes. Yet, the diabetes and the related complications continued to be major medical problem all over the world. The prevalence of diabetes mellitus is estimated to be more than 300 million by 2025. The WHO has recommended the evaluation of traditional plant treatments for management of diabetes as they are effective,

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less toxic with minimum or no side effects and are considered to be excellent candidates for oral therapy<sup>9</sup>. Many herbs are used to treat metabolic disorders, cardiovascular problems, liver disorders, central nervous system and digestive system related disorders. The disease is a major degenerative ailment in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders<sup>10</sup>. Diabetes mellitus is one of the common metabolic disorders and 1.5% of the total population suffers from this disease throughout the world<sup>11</sup>.

*Lepidagathis cristata* (Acanthaceae) popularly commonly known as “Nakka pintuka”, is a perennial herb which don't have stem. The plant parts are being used in Indian folk medicine. The leaves were reported to be useful to treat malarial fever, stomachic, itch, scabies, skin abscess and tumours<sup>12</sup>. However, the above plant is claimed to possess anti diabetic activity, but no scientific evidence is supported. Therefore, study of anti diabetic effect of *Lepidagathis cristata* was undertaken to evaluate the potential of the activity against alloxan induced diabetic rats.

## 2. MATERIALS AND METHODS:

### 2.1. Materials

Drugs: Glibenclamide, Normal saline, Alloxan monohydrate were used in this study.

### 2.2. Plant material

The leaves of *Lepidagathis cristata* were collected during flowering season in the month of March at S.V.U campus in Tirupati. The plant was authenticated by Dr. Madhava Chetty, Taxonomist, S.V. University, Tirupati, and voucher specimen was numbered and deposited in our Research lab P.R.R.M. College of Pharmacy.

### 2.3. Extraction

The fresh leaves were collected, washed with tap water, shade dried for two weeks pulverized, sieved (10/44) and stored in air-tight containers. About 1kg of powdered drug was extracted with ethanol by using soxhlet apparatus until the phyto constituents were completely exhausted. The ethanolic extract was evaporated through rotary evaporator (Buchi type, Mumbai, India) under reduced pressure at 40°C and labelled.

## 2.4. Phytochemical investigation

Phytochemical analysis of ethanolic extract was carried out to find out the presence of various phytoconstituents i.e., flavonoids, glycosides, phenolics, carbohydrates, tannins, triterpenoids, saponins etc<sup>13</sup>.

## 2.4. Experimental animals

Wistar rats (200-250gms) of either sex selected from animal house, supplied by National Institute of Nutrition, Hyderabad, India. The animals were housed under standard laboratory conditions maintained at 25 ± 10°C under 12/12 h light dark cycle and fed with standard pellet diet and water *ad libitum*. The experimental protocol was approved by the institutional animal ethics committee and by the animal regulatory body of the Indian Government.

## 3. ACUTE TOXICITY STUDIES

The acute oral toxicity test of the extract was determined according to OECD (Organisation for Economic Co-operation and Development). The studies were performed according to OECD Guidelines 425.

## 4. EXPERIMENTAL DESIGN

### 4.1. Diabetes induction:

The induction of diabetes was done by using Alloxan monohydrate in the dose of 120mg/kg body weight dissolved in normal saline and given intraperitoneally to overnight fasted animals. The rats were kept for the next 24hrs on 10% of glucose solution in water bottles to prevent hypoglycaemia and death. After 72 hrs of injection, fasting blood glucose levels were measured. The animals that did not show blood glucose levels more the 250mg/dl were rejected<sup>14, 15</sup>.

### 4.2. Oral Glucose Tolerance Test for EELC

Wistar rats of either sex were divided into six groups with each group containing six animals. Group I animals served as normal and received glucose (2g/kg), animals in group II, received standard drug of glibenclamide (5 mg/kg) with glucose (2 g/kg). Animals in group III were treated with test EELC (100 mg/kg) and glucose (2 g/kg), group IV was treated with test EELC (200 mg/kg) and glucose (2 g/kg), and Group V was treated with test EELC (400 mg/kg) and glucose (2 g/kg).

The animals were fasted overnight and treated with above dosage schedule orally. The EELC and glibenclamide were administered half an hour before administration of glucose solution.

#### **4.3. Anti diabetic Activity of *Lepidagathis cristata*:**

##### **Grouping:**

In experiment, the rats were divided into following groups with six animals in each group.

Group- I: Normal control rats fed with 0.5 ml of normal saline.

Group-II: Diabetic control rats fed with 0.5ml of normal saline.

Group-III: Diabetic rats treated with standard drug Glibenclamide 5mg/kg b.w.

Group-IV: Diabetic rats treated with ethanolic extract of *L.cristata* 100mg/kg.b.w.

Group-V: Diabetic rats treated with ethanolic extract of *L.cristata* 200mg/kg.b.w.

Group-VI: Diabetic rats treated with ethanolic extract of *L.cristata* 400mg/kg.b.w.

The treatment was given once a day for 21 days. The normal and control group received an equal volume of vehicle.

#### **4.4. Biochemical analysis**

Blood samples were collected from the animals prior to the treatment with above schedule and after 30 min of glibenclamide/ethanol extracts administration on 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day. Blood was obtained from the retro orbital venous plexus of rats under ether anaesthesia using a glass capillary tube and was centrifuged (2,500 rpm/10 min) to separate serum. The serum was used for biochemical analysis of blood glucose, TC, TG, HDL, LDL, SGOT, SGPT and ALP.

#### **4.5. Collection of organs and analysis**

After 21 days all the animals were euthanized by overdose of ether anaesthesia. The glucose levels were estimated by commercially available glucose kits (Span Diagnostics Ltd, Surat, India) based on glucose oxidase method, TC, TG, HDL, LDL and SGOT, SGPT, ALP<sup>16,17</sup>.

#### **4.6. Statistical analysis**

Data was expressed as mean  $\pm$  SEM, (n=6). Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. Values were considered statistically significant when at  $p < 0.05$ .

## **5. RESULTS and DISCUSSION**

### **5.1. Phytochemical studies**

As per the results of phytochemical study, *L.cristata* have exhibited the presence of flavonoids, glycosides, carbohydrates, tannins, saponins and mucilages in appreciable amounts. The results are given in Table 1.

### **5.2. Effect on oral glucose tolerance**

The blood glucose levels in the control group (Group-I) were found to increase maximum levels within 30 min after glucose load and normal glucose levels were observed over a period of 100 min. In Group-II (glibenclamide treated group) and Group-VI (400 mg/kg, treated group) the blood sugar levels returned to normal within 30 min. Group III, IV, and V showed significant decrease in blood glucose levels at 90 min. Results in table 2, suggested that the *L.cristata* have not decreased the blood glucose levels below normal levels.

### **5.3. Biochemical Analysis**

As of Table 5, groups G-IV to G-VI (100,200 and 400 mg/kg) have shown a dose dependent decrease in the serum glucose levels on 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day. G-VI showed equipotent activity with G-III. The *L. cristata* (100, 200 and 400 mg/kg) have shown a dose dependent decrease in the serum glucose levels on 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day. G-VI showed equipotent activity with G-III. The *L.cristata* at the dose of 400 mg/kg showed an efficient anti diabetic activity and its efficacy was found to be on par with glibenclamide. The plausible mechanism behind the anti diabetic potential of *L.cristata* could be due to the presence of glycosides, flavonoids and tannins which would have increased the activity of enzymes responsible for utilization of glucose by insulin-dependent pathway. Lowering of blood glucose level in alloxonised rats after administration of the extracts indicated that the extracts possessed extra-pancreatic effects or regeneration of  $\beta$ -cells in pancreatic islets<sup>18-22</sup>. Lipid profile experimental results of EELC at 400 mg/kg have shown a significant decrease in TG, TC, LDL, SGOT, SGPT and ALP and the progressive decrease in lipid levels were observed over 21 days period of treatment which was in dose-dependent manner and also noted during the study period.

The devoid levels of lipids in serum could be due to the erratic affects of lipolytic compounds on adipose matter, majorly due to insulin. In general conditions, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. Hyper triglyceridemia and hyper cholesterolemia caused due to inactivation of lipoprotein lipase in insulin deficiency<sup>23,24</sup>. The altered serum lipid profile was returned to normal after treatment with ethanol extracts. From the results obtained, that both ethanol extracts i.e., *L.cristata* have shown effective antidiabetic activity against alloxan induced diabetic wistar rats at the dose of 400 mg/kg. For comparison, the ethanol extract of *L.cristata*, have exhibited a potential anti diabetic activity on long-term treatment in Wistar rats. It could be due the presence of relatively more amounts of glycosides, carbohydrates flavonoids, saponins, tannins and saponins. In conclusion, *L.cristata* have demonstrated a significant anti-diabetic potentials and which could be via restoration of

the pancreatic functions, activation of the beta cells, decreased absorption of glucose and/or by phytochemical contents. However, further studies are required to assess the antioxidant properties, isolation and characterization of the bioactive compounds/enzymes responsible for anti diabetic activity and the establishment of the exact mechanism(s) of action.

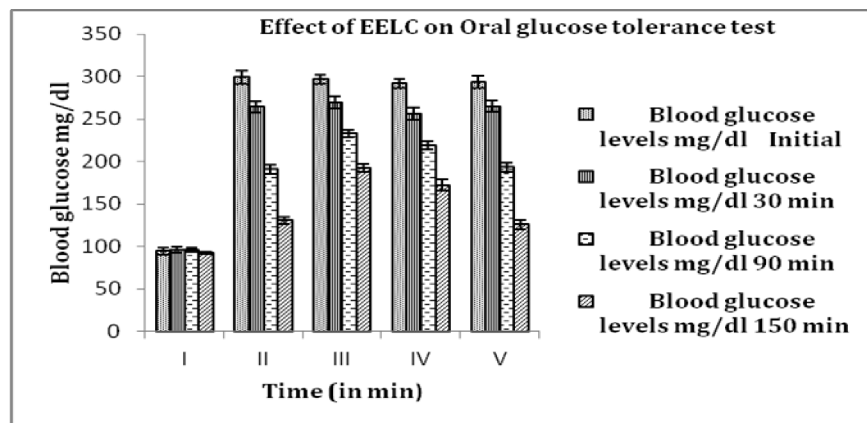
**Table 1:** Qualitative phytochemical determination of active ingredients in crude extract of *L.cristata*

Plant constituents	Ethanollic extract
Flavonoids	Positive
Carbohydrates	Positive
Triterpenoids	Positive
Glycosides	Positive
Tannins	Positive
Saponins	Positive
Mucilages	Positive
Alkaloids	Negative
Proteins and aminoacids	Negative

**Table 2:** Effect of EELC on Oral glucose tolerance test

Group	Blood glucose levels mg/dl			
	Initial	30 min	90 min	150 min
I	94.4±4.3*	96.24±3.5**	95.4±2.4*	92.4±1.6*
II	298.8±8.1*	264.5±6.6*	190.5±5.6*	130.5±4.4*
III	296.6±5.3*	269.4±7.3*	232.8±4.2**	192.1±4.6**
IV	292.3±5.4*	255.8±7.4**	218.6±4.5*	172.2±6.7*
V	293.5±6.8*	264.9±6.9*	193.4±5.5*	125.5±5.6*

Each value represents the mean ± SEM. n = 6, number of animals in each group. Values \*\*P<0.01, \*P<0.05. Compared to positive control.

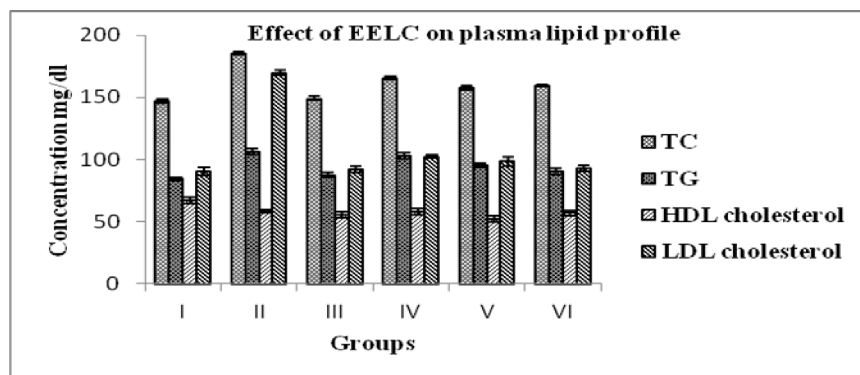


**Table 3:** Effect of EELC on plasma lipid profile

Groups	TC (mg/dl)	TG (mg/dl)	HDL cholesterol (mg/dl)	LDL Cholesterol (mg/dl)
I	146.67	84.2	67.41	90.26
II	185.4	106.3	58.34	169.61
III	148.62(↓19.83%)	87.4(↓17.77%)	55.67(↓4.576%)	91.86(↓45.84%)
IV	165.4(↓10.78%)	102.6(↓3.480%)	58.04(↓0.514%)	102.35(↓39.65%)
V	157.5(↓15.04%)	95.2(↓10.44%)	52.27(↓1.834%)	98.27(↓42.06%)
VI	159.4(↓14.02%)	90.5(↓14.86%)	56.86(↓2.536%)	92.75(↓45.31%)

All the values are shown as Mean±SEM, n=6.

**Fig 3:** Effect of EELC on plasma lipid profile

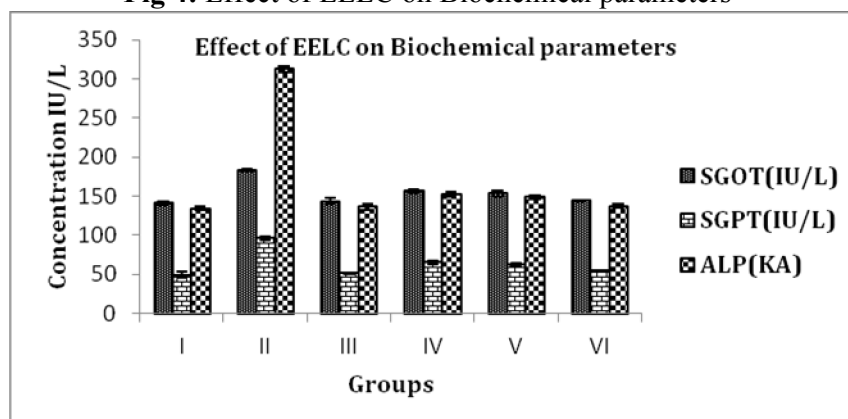


**Table 4:** Effect of EELC on Biochemical parameters

Groups	SGOT(IU/L)	SGPT(IU/L)	ALP(KA)
I	141.26	49.17	134.23
II	182.7	96.7	312.25
III	143.25(↓21.59%)	51.26(↓46.99%)	136.36(↓56.32%)
IV	156.91(↓14.11%)	65.26(↓32.51%)	152.3(↓51.22%)
V	153.27(↓16.10%)	62.35(↓35.52%)	149.25(↓52.20%)
VI	144.21(↓21.06%)	54.25(↓43.89%)	137.23(↓56.05%)

All the values are shown as Mean±SEM, n=6.

**Fig 4:** Effect of EELC on Biochemical parameters



## 6. DISCUSSION:

Diabetes mellitus is the name given to a group of disorders characterized by chronic Hyperglycaemia, polyuria, polydipsia, polyphagia and weakness due to disturbance in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion and its action. These metabolic deregulations leads to secondary patho physiological changes in multiple organ system including both micro & macro vascular dysfunctions<sup>25</sup>. The treatment strategies mainly include nutritional therapy, oral hypoglycaemic agents, insulin preparations and or combination of any of these strategies<sup>26</sup>. In this study, evaluated the effect of *L.cristata* leaves extract on fasting blood glucose estimation done in diabetic induced wistar rats. Diabetic induction was done by administrating alloxan monohydrate in the dose of 120mg/kg body wt by intra-peritoneal route. This chemical induces necrosis to islets  $\beta$ -cells through free radical mediated damage, thus producing partial destruction of pancreatic beta cells, so insulin deficiency will lead to marked increase in blood glucose level producing type 2 DM<sup>27</sup>. After stabilization of hyperglycaemia the study has been conducted for 21 days. Oral treatment with an ethanolic extract of leaves of *L. cristata* has produced significant fall in fasting blood glucose levels. The extract of leaves of *L. cristata* at the dose 400mg/kg has produced a highly significant decrease (p value < 0.001) in blood glucose levels as compared to the doses of *L.cristata* 100mg/kg and 200mg/kg body wt. Many traditional plant treatments for diabetes mellitus are using throughout the world. Few of the traditional plants treatments for diabetes have received scientific scrutiny as in the WHO has recommended<sup>28</sup>. Like *Trigonella foenum graecum*, *Momordica charantia*, *Tinospora cordifolia*, *Enicostema littorae*, *Gymnema sylvestre*, *Azadirachta indica*, *Syzi-gium cumini* are some of the most effective and the most commonly studied Indian plants in relation to diabetes<sup>29,30</sup>.

Increased serum concentration of qualitative diagnostic enzymes such as SGOT, SGPT and ALP were observed in diabetic rats indicating an altered liver function and/or liver mitochondrial injury in comparison to normal control rats. Insulin deficiency contributes to

increased serum level of transaminase enzymes due to easily availability of amino acids which leads to enhanced occurrence of gluconeogenesis and ketogenesis processes during diabetes. On treatment with EELC (400mg/kg) significantly reversed the elevated marker enzymes i.e., SGOT, SGPT, ALP and restored to normal values indicates a revival of insulin secretion into circulations and also its hepato protective effect. A marked increase in serum concentration of TC, TG, LDL and decreased HDL was obtained with diabetic rats than normal group which is often linked with hyperlipidemia.

In this study, the possible mechanism by which *L.cristata* leaves bring about its hypoglycaemic action may be by potentiation of pancreatic secretion of insulin from  $\beta$ -cell of islets or due to enhanced transport of blood glucose to peripheral tissue and also presence of tannins, flavonoids, glycosides, saponins, and triterpenoids, carbohydrates etc. The EELC found to be potential anti diabetic extract in alloxan induced diabetic model through reducing oxidative damage and modulating antioxidant enzymes by dose dependant manner.

## 7. CONCLUSION

In conclusion, *L.cristata* leaves extract at dose 400mg/kg body weight and glibenclamide showed significant hypoglycaemic activity in diabetic rats after oral administration. Thus, the present observation provide evidence that ethanolic extract of *Lepidagathis cristata* leaves exhibited anti diabetic or hypoglycaemic activity on alloxan induced diabetic rats may be due to enhancing the peripheral utilization of glucose by correcting the impaired liver or kidney glycolysis and by suppression of its gluconeogenic activity similar to insulin. This effect may be due to the presence of flavonoids, triterpenoids, glycosides, tannins, saponins and mucilages and other constituents present in the leaves which could act synergically or independently in enhancing the activity of glycolytic and gluconeogenic enzymes. However, Further, comprehensive chemical and pharmacological investigation should be carried out to isolate the active compound and appropriate elucidation of its mechanism of action.

The result suggests that it is worth undertaking further studies on possible usefulness of the *Lepidagathis cristata* leaves in diabetes mellitus.

## 8. ACKNOWLEDGEMENT

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