

## Anti-Diabetic Activity of Aqueous extract of aerial parts of *Allamanda Cathartica* Linn in Diabetic Rats Induced by Alloxan

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### Abstract

Diabetes is a group of diseases marked by high levels of blood glucose, also called blood sugar, resulting from defects in insulin production, insulin action, or both. In modern medicine no satisfactory effective therapy is yet available to cure diabetes mellitus. Though several oral hypoglycaemic agents are used for the management of diabetes mellitus, there are several drawbacks. They are also not approved for the treatment of women who are pregnant with diabetes. Thus, alternative therapy is required. So the search for newer anti-diabetic drugs with minimum or no side effects from herbal plants is a challenge as per WHO recommendations.

The present study was aimed to evaluate the anti-diabetic potency of *Allamanda cathartica* on the blood glucose level in alloxan induced diabetic rats. Diabetic Albino Wistar strain rats were treated with standard drug Glibenclamide and test drug *Allamanda cathartica* at 200mg (low dose) and 400mg (high dose). The hypoglycemic effect was determined in the rats and the efficacy of the test drug was compared to the standard drug Glibenclamide. *Allamanda cathartica* was orally administered for 28 days in alloxan induced diabetic rats. At the end of the study duration, blood glucose level and body weight were statistically analyzed. Based on these results of the study *Allamanda cathartica* produced a significant reduction in blood glucose levels, serum enzymes (SGPT and SGOT) and slight increase in the body weight when compared with diabetic control rats. Hence the present research work proved that the *Allamanda cathartica* possess hypoglycemic effect.

**Keywords:** Diabetes mellitus, Alloxan, Glibenclamide, *Allamanda cathartica*, Blood glucose level, Body weight, Anti-diabetic activity.

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### Introduction

Carbohydrates from the diet are the primary exogenous source of glucose. Glucose is the main fuel for energy requirement of the body. The impairment in glucose metabolism therefore, may lead to physiological imbalance and warrants proper management. Any variation therefore, in normal glucose metabolic pathway may lead to the impaired glucose metabolism, the onset of hyperglycemia and subsequently diabetes mellitus.<sup>(1,2)</sup> So this a deficiency of insulin result in improper metabolism of glucose which have harmful effect in the body system, in particular the blood vessels and nerves.<sup>(3)</sup> According to WHO recent estimation, approximately 285 million people worldwide (6.6%) in the 20–79 year age group will have diabetes in 2010 and by 2030, 438 million people (7.8%) of the adult population, is expected to have diabetes. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030.<sup>(4)</sup>

In modern medicine no satisfactory effective therapy is yet available to cure diabetes mellitus. Though several oral hypoglycemic agents are used for the management of diabetes mellitus, there are several drawbacks like high costs, weight gain, head ache, cardiac complications, anorexia, nervosa, brain atrophy and fatty liver.<sup>(5)</sup> They are also not approved for the treatment of women who are pregnant with diabetes. Thus, alternative therapy is required.

Literature survey revealed that, the aerial parts of *Allamanda cathartica* possess anti-diabetic property.<sup>(6)</sup> Hence, the present study is designed to evaluate the efficacy of aerial part extract of *Allamanda cathartica* on experimental models of diabetes using rats.

### Materials and Method

The fresh plants of *Allamnda cathartica linn* were collected in the month of july-august from the local areas of Mangalore district, Karnataka state, India. The taxonomic were authenticated by Ms Aparna Upadhyaya, Botanist, Teacher, government high school, Hodavada, Madikeri, Karnataka. The aerial parts were shade dried, the dried aerial parts were pulverized into coarse powder at plant mill and sieved by using a mesh no. 10/44 and 100g of the powered samples were extracted using aqueous solvent in a soxhlet apparatus. The extracts obtained were dried and used for anti-diabetes studies.

**Experimental Animals:** Healthy Wistar albino rats (150–200g) of either sex were used for the experiment were procured from the animal house of Srinivas College of Pharmacy, Mangalore. They were maintained under standard conditions (temperature 22 ± 2°C, relative humidity 60±5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. All the experimental protocols were reviewed and approved by the institutional animal

ethical committee (Approval no SCP/IAEC/F150/P91/2016) prior to the initiation of the experiment and the care of the laboratory animals were taken as per the CPCSEA regulations. The animals were acclimatized for atleast one week before use.

**Acute Toxicity Evaluation:**<sup>(7)</sup> Acute toxicity study of the aqueous extract of *Allamanda cathartica* (ACAE) were performed as per the OECD guidelines 425 at a limit dose of 2000 mg/kg. The doses were administered by oral route in albino mice (20-25g). Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for total 14 days for sign of toxicity and/or mortality if any.

**Experimental Design:**<sup>(8)</sup> The Wistar albino rats (150-200g) of either sex were randomly divided into five groups of six each. The different groups were assigned as follows.

Group I: Normal control (Vehicle)

Group II: Diabetic control (Alloxan100 mg/kg)

Group III: Diabetic animal(Alloxan100 mg/kg + Glibenclamide 5mg/Kg )

Group IV: Diabetic animals (Alloxan100 mg/kg + ACAE 200mg/kg)

Group V: Diabetic animals (Alloxan100 mg/kg + ACAE 400mg/kg)

**Treatment:** All the animals except group I were made diabetic by a single intra peritoneal injection of alloxan (100mg/kg body weight) in normal saline. After two days of alloxan injection the blood glucose level was assessed and the animals having blood sugar level >200

were selected for the study. All the treatment was given orally once daily for entire 30 days.

**Evaluation:** Starting from the first day of treatment, blood was collected every week from retro orbital puncture and glucose level was estimated by using Accu-Chek Active glucose monitoring kit.

**Biochemical Analysis:** The animals were sacrificed at the end of experimental period of 28 days by decapitation. Blood was collected, sera separated by centrifugation at 3000 rpm for 10 minutes. serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) levels in the normal, diabetic control and drug treated rats was measured spectrophotometrically as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit using Semi Autoanalyser.

**Statistical Analysis:** The results were represented as Mean  $\pm$  SD. The statistical significance was computed using One Way ANOVA followed by Tukeys multiple comparison test and compared with diabetic control group with Standard drug.

**Methods for estimation of Biomarkers:** The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit using Semi Autoanalyser.

**Histopathological Studies:** Pancreas will be allowed to fix in 10% formalin. Washed in running water followed by dehydration with isopropyl alcohol and impregnated with paraffin wax. Section will be made using microtome. After staining with eosin, the different histopathological indices will be determined.

## Results

**Table 1: Effect of *Allamanda cathartica* aqueous extract on blood glucose level in Alloxan induced diabetic rats**

Groups	Blood glucose level(mg/dl)				
	Initial	Day 7	Day 14	Day 21	Day 30
Normal control	85.00 $\pm$ 1.125	86.33 $\pm$ 0.6146	87.00 $\pm$ 0.6831	81.67 $\pm$ 1.430	81.17 $\pm$ 1.078
Diabetic control	324.8 $\pm$ 4.362	338.3 $\pm$ 5.625	341.5 $\pm$ 4.493	332.0 $\pm$ 6.894	317.7 $\pm$ 14.10
Glibenclamide (5mg/kg)	326.2 $\pm$ 4.629	258.0 $\pm$ 14.33**	201.2 $\pm$ 15.69***	142.7 $\pm$ 13.57***	102.8 $\pm$ 4.086**
ACAE (200mg/kg)	320.7 $\pm$ 3.499	267.3 $\pm$ 17.72*	223.3 $\pm$ 14.37*	185.7 $\pm$ 12.26*	142.2 $\pm$ 10.63*
ACAE (400mg/kg)	324.5 $\pm$ 4.843	263.7 $\pm$ 17.72**	215.3 $\pm$ 13.32**	163.5 $\pm$ 8.543**	118.0 $\pm$ 3.958**

Values are expressed as mean  $\pm$  S.E.M, n=6 in al group except in diabetic control One way ANOVA followed by Dunette's t- test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, when compared with diabetic control rats.

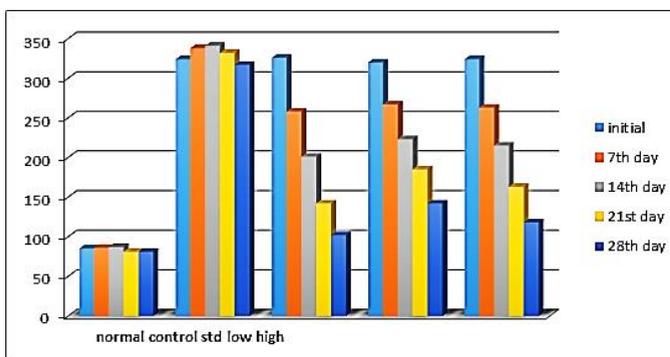


Fig. 1: Effect of ACAE on blood glucose level in Alloxan induced diabetic rats

Table 2: Body weight in alloxan induced diabetic rats

Groups	Body Weight (Grams)			
	Day 0	Day 7	Day 14	Day 28
Normal control	176.2± 0.7491	191.7± 0.7149	196.0± 3.173	208.8± 1.014
Diabetic control	182.5± 0.8062	163.2± 0.7032	152.7± 1.626	139.3± 1.085
Glibenclamide (5mg/kg)	186.2± 0.6540**	181.0± 0.5264***	191.5± 0.5627**	196.7± 1.022***
ACAE (200mg/kg)	201.0± 0.5164*	195.0± 1.414*	201.2± 2.561*	205.8± 2.040*
ACAE (400mg/kg)	200.5± 0.3416*	192.5± 1.147**	201.0± 0.4472*	209.2± 0.4014**

Values are mean ±SEM (n=6) one way ANOVA followed by Dunette's test. Where, # represents the comparison, \* represents significant at p<0.05, \*\* represents highly significant at p<0.01 and \*\*\* represents very significant at p<0.001.

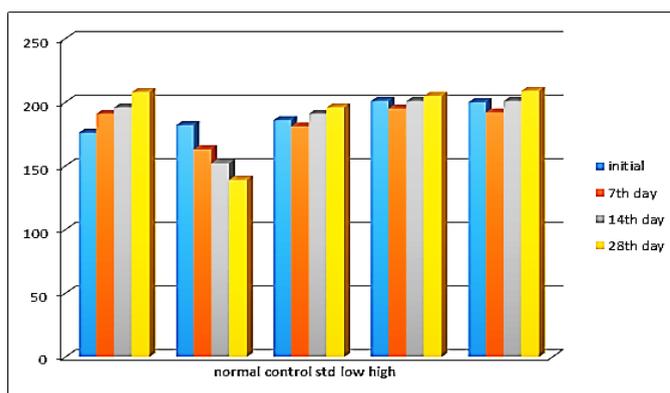
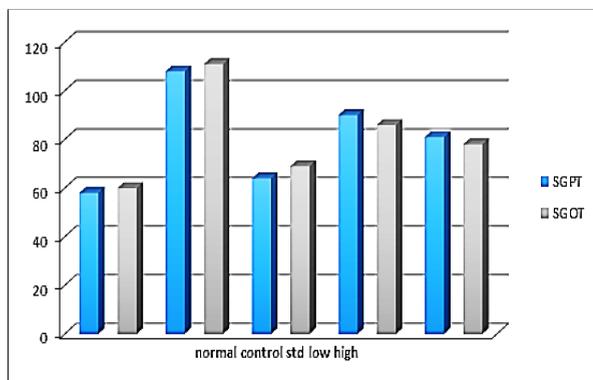


Fig. 2: Effect of ACAE on animal body weight in Alloxan induced diabetic rats

Table 3: SGPT and SGOT levels in Alloxan induced diabetic rats

Group	Alloxan	
	SGPT	SGOT
Normal control	58.00± 0.3651	60.17± 0.6009
Diabetic control	108.7± 0.4944	111.0± 0.3651
Standard Glibenclamide	64.83± 0.3073***	69.17± 0.4773***
ACAE (200mg/kg)	90.17± 0.3073*	86.17± 0.4773*
ACAE (400 mg/kg)	81.50± 0.4282**	78.00± 0.3651**



**Fig. 3:** Effect of ACAE on SGPT and SGOT level in Alloxan induced diabetic rats

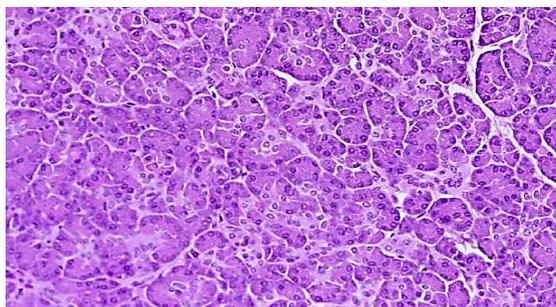
### Histopathology studies of pancreas in both alloxan and STZ induced diabetic rats:

**Normal control:** normal rat showing normal acini and normal cellular population in islets of Langerhans and absence of both damage to islets and hyperplasia (Fig. 4)

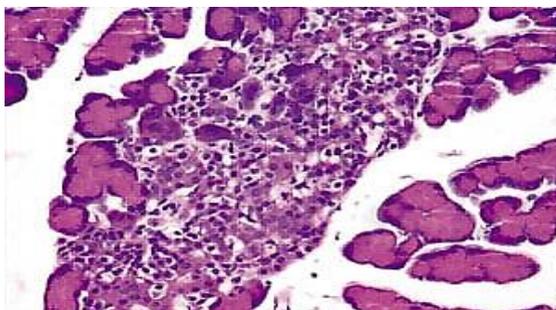
**Standard:** diabetic rats treated with GLB 5mg/kg showing complete restoration of normal cellular population size of islets of Langerhans and absence of islet damage and presence of hyperplasia. (Fig. 5) in Alloxan

**ACAЕ treated in Alloxan induced diabetes:** ACAЕ 400 mg/kg showing restoration of normal cellular population size of islets of Langerhans and cells are partially preserved (Fig. 6)

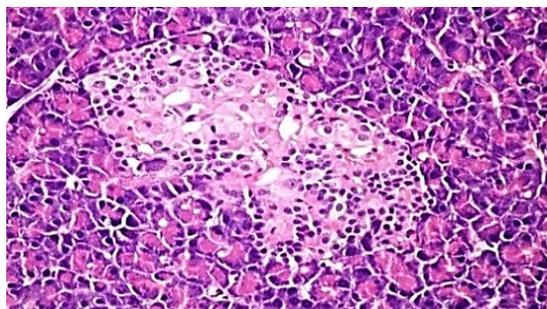
**Diabetic control:** it suggested extensive damage to the islets of Langerhans and reduced islet size. (Fig. 7) in Alloxan.



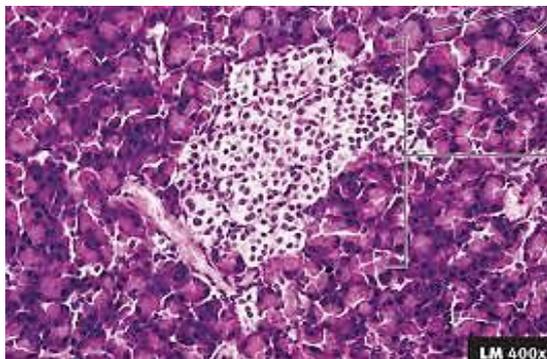
**Fig. 4:** Normal control



**Fig. 5:** Glibenclamide(alloxan)



**Fig. 6:** ACAE 400mg/kg (Alloxan)



**Fig. 7:** Diabetic control(Alloxan)

### Results and Discussion

The commonly used chemical agent in laboratories for inducing diabetes in animal is alloxan which is an oxidized product of uric acid that causes destruction of beta cells of the pancreas by oxidation mechanism and produce Type 1 diabetes. The present study screened the anti diabetic activity of the *Allamanda cathartica* against alloxan induced diabetic rats. The continuous treatment of the *Allamanda cathartica* extract was done for a period of 28 days at 200mg/kg and 400 mg/kg of body weight. Glibenclamide was the standard drug used to stimulate insulin from beta cells of islets of langerhans many years in research. So, Glibenclamide (5mg/Kg) was selected as standard drug in the study. The results of the blood glucose level and body weights of the normal control group, diabetic control group, standard group (Glibenclamide 5mg/kg) and *Allamanda cathartica* were summarized in Table 1 and 2. Data are statistically obtained by using one way ANNOVA followed by Tukeys multiple comparison test.

In Table 2, the body weight of the normal control is increased after 28days. However in diabetic control group was decreased from  $182.5 \pm 0.80$  to  $139.3 \pm 1.08$  after 28 days. The body weight of standard control group is slightly increased after 28 days of treatment. The initial body weight of ACAE (200mg/kg) group is  $201.0 \pm 0.51$ , and after 28 days of treatment the body weight was about near to  $205.8 \pm 2.04$ , and in ACAE (400mg/kg) initial body weight found to be  $200.5 \pm 0.34$  and after 28 days of treatment body weight was near to  $209.2 \pm 0.40$  there was slight increase in body weight found when compared with diabetic control group.

The effect of *Allamanda cathartica* aqueous extract on blood glucose level was studied in the animals. The test group showed a significant decrease in blood glucose level on alloxan induced diabetic rats when compared to diabetic control group. The initial reading of blood glucose level of ACAE (200 mg/kg) was  $324.5 \pm 4.843$  before treatment. After the 28 days period ACAE produced significant reduction in the blood glucose levels  $142.2 \pm 10.63$ . The initial reading of blood glucose level of ACAE (400 mg/kg) was  $324.5 \pm 4.843$  before treatment. After the 28 days period ACAE produced significant reduction in the blood glucose levels  $118.0 \pm 3.95$ . In standard drug group initial blood glucose level was  $326.2 \pm 4.629$  and the after 28 days it was  $102.8 \pm 4.08$  which showed that the standard drug had produced maximum anti-diabetic effect. The diabetic control group showed rise in blood glucose level throughout the study period. Initially the blood glucose level of diabetic control group was  $324.8 \pm 4.362$  and after 28 days of study period the blood glucose level was increased to  $317.7 \pm 14.10$ . The results of blood glucose level in rats were summarized in Table No.1. And on the basis of the results, it was observed that there was a significant reduction in blood glucose level by *Allamanda cathartica* aqueous extract in alloxan induced diabetic rats.

*Allamanda cathartica* extract showed dose dependent significant decrease in biochemical parameter such as SGPT and SGOT level when compared with diabetic control. The results of SGPT and SGOT in rats were summarized in Table 3. Histopathology of pancreas showed regeneration of  $\beta$ -cell in extract treated diabetic rats.

The anti-diabetic activity of ACAE could be due to the increased release of insulin from beta cells of the pancreas or may be due to potentiating effect of insulin. Treatment of ACAE in diabetic rat also showed the significant weight gain property which proved its efficacy of this Polyphyto mixture in treating diabetic patients successfully.

## Conclusion

Aqueous extract of *Allamanda cathartica* aerial part is found to be more effective in the treatment of diabetes mellitus as determined by its statistically significant p-value  $< 0.001$  in alloxan induced diabetic rats. The mechanism of anti-diabetic activity of ACAE may be due to enhancing the effect of insulin and by stimulating the insulin secretion from beta cells of pancreas. Hence this study suggests that *Allamanda cathartica* aqueous extract has a potent anti diabetic effect which could be used for the management of diabetes effectively.

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