Research Article

An Investigation on Anti-Diabetic Activity in Aqueous Extract of Aerial Parts of *Allamanda cathartica Linn* in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus (DM) consists of a group of syndromes characterized by hyperglycemia. Prevalence of both type 1 and type 2 DM is increasing worldwide, the prevalence of type 2 is rising much more rapidly because of increasing obesity and reduced activity levels as countries become more industrialized. *Allamanda cathartica* a medicinal plant widely used in the traditional Ayurveda and Siddha systems of medicine for the treatment of diabetes mellitus. In the present study the antidiabetic potential of *A.cathartica* aqueous extract (ACAE) was evaluated in the Streptozotocin (STZ) - induced type 2 diabetic models. The dose 200 mg/kg and400 mg/kg of ACAE were administered to normal and experimental diabetic rats for 28 days. The significantly ($p< 0.05$) reduction in blood glucose levels were observed in the std and ACAE treated diabetic animals at 28 days, Normal control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during treatment period. SGPT and SGOT level were reduced in a dose dependent manner and body weight improves by the extracts at dose of 200mg/kg and 400mg/kg during treatment period. And hence the present research work proved that the *Allamanda cathartica* possess hypoglycemic effect in experimental animals.

Keywords: Diabetes mellitus, streptozotocin, Glibenclamide, Allamanda cathartica, Body weight, Anti-diabetic activity.

INTRODUCTION

Diabetic mellitus (DM) is the condition arising due to abnormal metabolism of carbohydrate, proteins and fats. It is caused by insulin deficiency, often combined with insulin resistance. This disorder occurs worldwide and its occurrence is increasing quickly in most of the countries. The treatment of DM is based on parenteral insulin and oral anti-diabetic drugs. Oral hypoglycemic agents, currently used have serious side effect hence there is a need to search a newer anti-diabetic agents that having high therapeutic efficacy with minimum side effect. This may be fulfilled by treating DM with traditional medicine using as anti-diabetic agents from medicinal plants. Diabetes mellitus (DM) consists of a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins; and an in-creased risk of complications from vascular disease. Though pathophysiology of diabetes remains to be fully understood, experimental evidences suggest the involvement of free radicals in the pathogenesis of diabetes and more importantly in the development of diabetic complications [1]

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 largest increases will take place in the regions dominated by developing economies. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030[2].

A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of NIDDM. Among these are alkaloids, glycosides, galactomannan gum, polysaccharides, peptidoglycans,

hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions. Thus, plants are a potential source of anti-diabetic drugs.

Although, oral hypoglycemic agents/insulin is the main stay of treatment of diabetes and is effective in controlling hyperglycemia, they have prominent side effects and fail to significantly alter the course of diabetic complications. As the knowledge of heterogeneity of this disorder increases, there is needed to look for more efficacious agents with lesser side effects. Though development of modern medicine resulted in the advent of modern pharmacotherapeutics including insulin, biguanides, sulfonylurea's and thiazolidinediones, there is still a need to look for new drugs as no drug (except strict glycemic control with insulin) has been shown to modify the course of diabetic complications.

Searching the safe and potent remedies from the herbal origin for the treatment of hyperglycemia has become most fascinating and desired area of research for the pharmacologist. In spite of tremendous strides in the modern medicine there is no effective remedy by which tight glycemic control is possible without adverse effects. Herbal drugs are mostly out of toxic or of less toxic with fewer side effects and relatively low costs compared to the synthetic drugs.

Allamanda cathartica L. (Apocynaceae) is also known as Alamanda big flower or thimble lady. Is one of the most studied species of the *Allamanda* genus and often it is found in tropical and subtropical regions as an ornamental shrub in gardens. Studies have indicated the potential anti-inflammatory, laxative, antioxidant, antibacterial, antifungal and invitro hepatoprotective properties of *Allamanda* flower extracts. In traditional medicine an infusion of the bark and leaves is used as a purgative. The leaf extract has displayed anti-inflammatory, antifertility potency in male, antimicrobial activity against multiple drug resistant clinical pathogen and also found to exhibit antioxidant activity, membrane stabilizing property and healing activities. Literature survey revealed that, the aerial parts of *Allamanda cathartica* possess anti-diabetic property[3]. Hence, the present study is designed to evaluate the efficacy of areal part extract of *Allamanda catharica* on streptozotocin induced diabetic rats.

MATERIAL AND METHODS

Collection of plant material

Allamanda cathartica plant material was collected from local areas of Mangalore, Karnataka, India. The taxonomic were authenticated by Ms Aparna Upadhyaya, Botanist, Teacher, government highschool, Hodavada, Madikeri, Karnataka. *Allamanda cathartica* aerial part was washed under tap water and were efficiently dried under shade for about one week and protected from deterioration.

Preparation of extract

Extraction was carried out by aerial parts using successive solvent extraction process (soxhlation apparatus). The aerial part powder (100 g) was extracted with water for 12 cycles. After completion of soxhlation process the liquid extract was collected and concentrated under reduced pressure below 50°C, until a soft mass obtained it was dried and kept in a desiccator.

Preliminary phytochemical screening[4]

About 50 mg of the solvent-free extract was stirred with little quantity of dilute HCl and then filtered. The filtrate was tested for presence of various phytochemical constituents such as alkaloids, Carbohydrates, Steroids, Proteins, Phenols, Tannins, Flavonoids, Glycosides, Gums, Saponins and terpenes.

Experimental Animals

Experiments were performed with male wistar rats, weighing about 180-220 g. The animals were housed in individual polypropy-lene cages under standard laboratory conditions of light, temperature (22 ± 1°C) and relative humidity for at least one week before the beginning of experiment, to adjust to the new environment and to overcome stress possibly incurred during transit. Animals were given standard rat pellets and drinking water *ad libitum.* The animals were fasted 12 hours before the conduct of experiment and during the experiment they were withdrawn from food and water. The experiments were planned after the approval of Institutional Animal Ethical Committee(Approval no SCP/IAEC/F150/P91/2016).

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling techniques were employed in this study. The animal were kept fasting for

overnight providing only water. Then the extracts (ACAE) were administered orally at the dose of 2000 mg/kg. The animals were observed for toxic symptoms and behavioral changes continuously for the first 4 hrs after dosing. Finally, the number of survivors was noted after 24 hrs. From the next day onwards, each day 1 hour the behavioral change, clinical symptoms or mortality was observed in the same animals for the next 14 days.

Induction of diabetes with Streptozotocin[5]:

All the animals except group I were made diabetic by a single intra peritoneal injection of Streptozotocin (50mg/kg body weight) in normal saline. After two days of streptozotocin injection the blood glucose level was assessed and the animals having blood sugar level >200 was selected for the study. All the treatment was given orally once daily for entire 30 days.

Experimental Design[6]:

The Wistar albino rats (150-200g) of either sex will be randomly divided into five groups of six each. The different groups will be assigned as follows.

Group I : Normal control (Vehicle)
Group II : Diabetic control (STZ 50 mg/kg)
Group III : Reference Standard (STZ 50 mg/kg + Glibenclamide 5mg/Kg)
Group IV: Diabetic animals (STZ 50 mg/kg +AEAAC low dose)
Group V: Diabetic animals (STZ 50 mg/kg + AEAAC high dose)

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. On 30th day, post treatment blood was collected; serum was separated and used for estimation of various biochemical parameters like body weight, fasting glucose, SGPT and SGOT.

STATISTICAL ANALYSIS

All data were expressed as Mean±SEM. The statistical significance between groups were compared using one way ANOVA, followed by Dunnett's (multiple comparison test). P value less than 0.05 was considered as statistically significant.

METHODS FOR ESTIMATION OF BIOMARKERS

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.

RESULTS

Table 1: Preliminary phytochemical screening of aqueous extract of aerial parts of *Allamanda cathartica*

Sl. No.	Test	Result
1.	Alkaloids	+ve
2.	Carbohydrates	+ve
3.	Flavonoids	+ve
4.	Glycosides	+ve
5.	Saponins	+ve
6.	Steroids	+ve
7.	Tannins	+ve
8.	Proteins	+ve
9.	Volatile oil	-ve

Table 2: Effect of *Allamanda cathartica* aqueous extract on blood glucose level in STZ induced diabetic rats

Groups	Blood glucose level(mg/dl)				
	Initial	7th day	14th day	21st day	30th day
Normal control	85.83± 0.47	86.67± 2.246	87.17± 1.621	87.33± 1.498	86.50± 1.176
Diabetic control	32717± 4.821	343.7± 6.591	349.5± 7.877	338.7± 8.939	311.7± 13.25
Glibenclamide (5 mg/kg)	335.5± 4.945	233.2± 11.06***	181.2± 12.37**	116.2± 6.172***	100.5± 2.377**
ACAE (200 mg/kg)	334.8± 4.354	283.3± 6.800**	222.7± 5.909*	166.5± 10.62*	131.0± 4.405*
ACAE (400 mg/kg)	338.7± 4.022	281.3± 4.088**	183.8± 9.428**	131.8± 3.429**	108.0± 2.352**
Values are expressed as mean ± S.EM, n=6 in all except in diabetic control, one way ANOVA followed by Dunette's test. *p<0.05, **p<0.01 and ***p<0.001 when compared to diabetic control.					

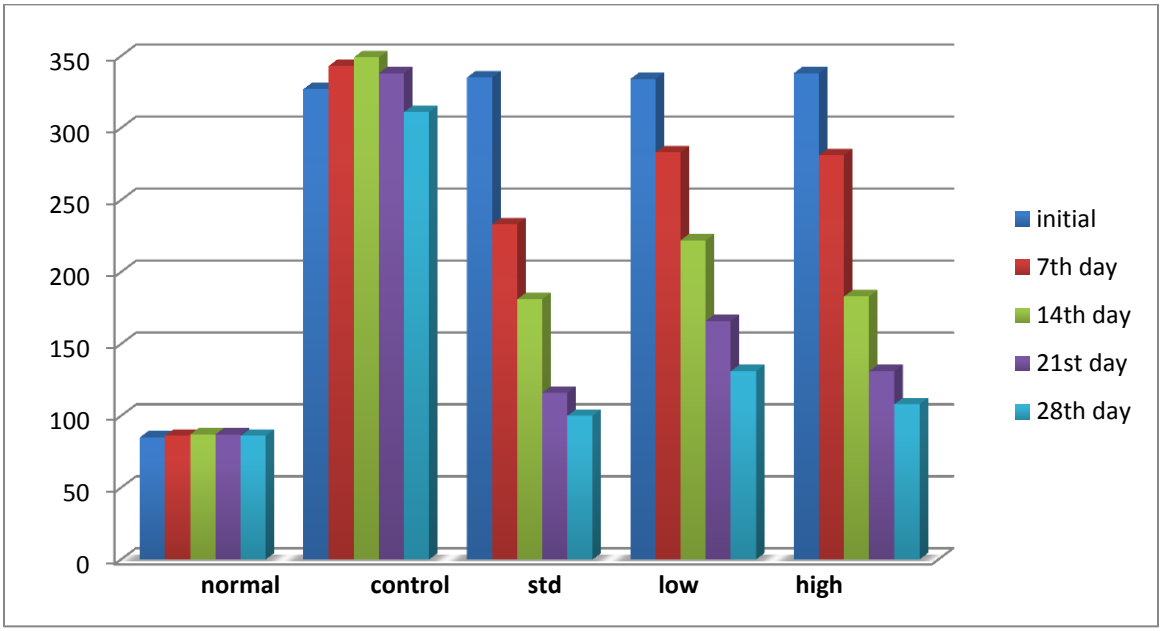

Fig. 1: Effect of ACAE on blood glucose level in STZ induced diabetic rats

Table 3: Body weight in STZ induced diabetic rats

GROUPS	BODY WEIGHT (Grams)			
	Day 0	Day 7	Day 14	Day 21
Normal control	176.2± 0.7491	191.7± 0.7149	208.8± 1.014	196.0± 3.173
Diabetic control	181.8± 0.8724	163.2± 0.7032	138.8± 0.7923	143.0± 1.506
Glibenclamide (5mg/kg)	177.0± 0.5164***	162.3± 0.333**	180.7± 0.333**	183.2± 1.579**
ACAE (200mg/kg)	202.5± 1.708*	179.7± 0.8433*	187.8± 1.195*	185.0± 0.8944*
ACAE (400mg/kg)	223.5± 1.708**	191.2± 1.1014*	210.7± 1.542*	203.7± 0.8819*
Values are mean ±SEM (n=6) one way ANOVA followed by Dunette's test. Where, # represents the comparision, * represents significant at p<0.05, ** represents highly significant at p<0.01 and *** represents very significant at p<0.001.				

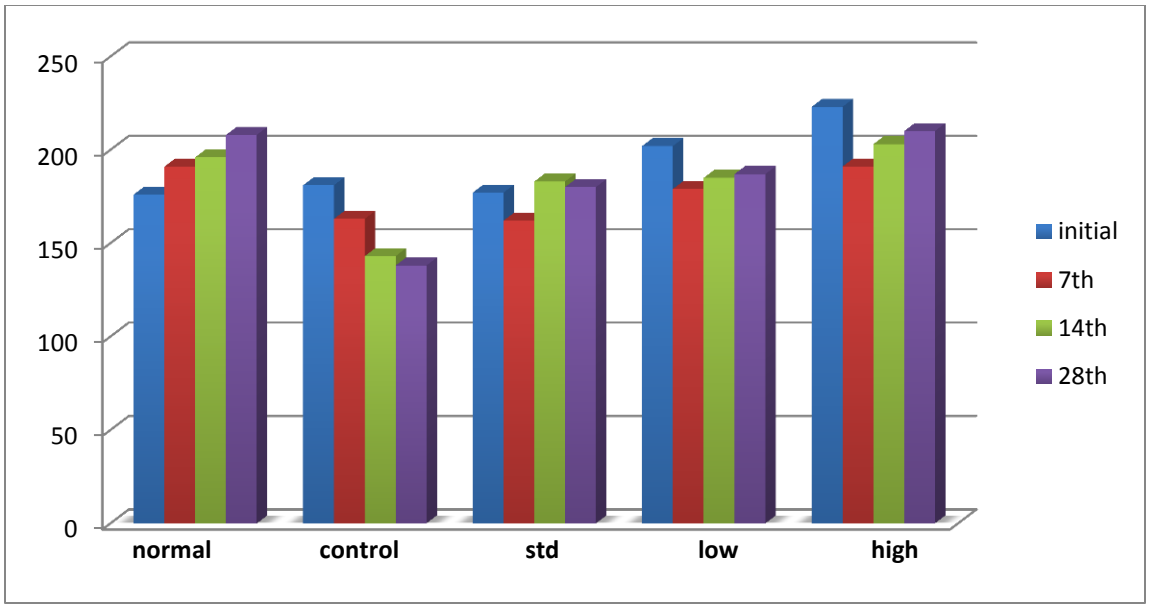

Fig. 2: Effect of ACAE on animal body weight in STZ induced diabetic rats

Table 4: SGPT and SGOT levels in diabetic rats

Group	STZ	
	SGPT	**SGOT**
Normal control	55.17± 0.3073	56.00± 0.3651
Diabetic control	100.3± 0.4216	111.5± 0.7188
Standard Gibenclamide	70.33± 0.7601***	68.33± 0.7149***
ACAE (200mg/kg)	97.83± 0.4773*	95.00± 0.5774*
ACAE (400 mg/kg)	84.67± 0.6146**	76.83± 0.4014**

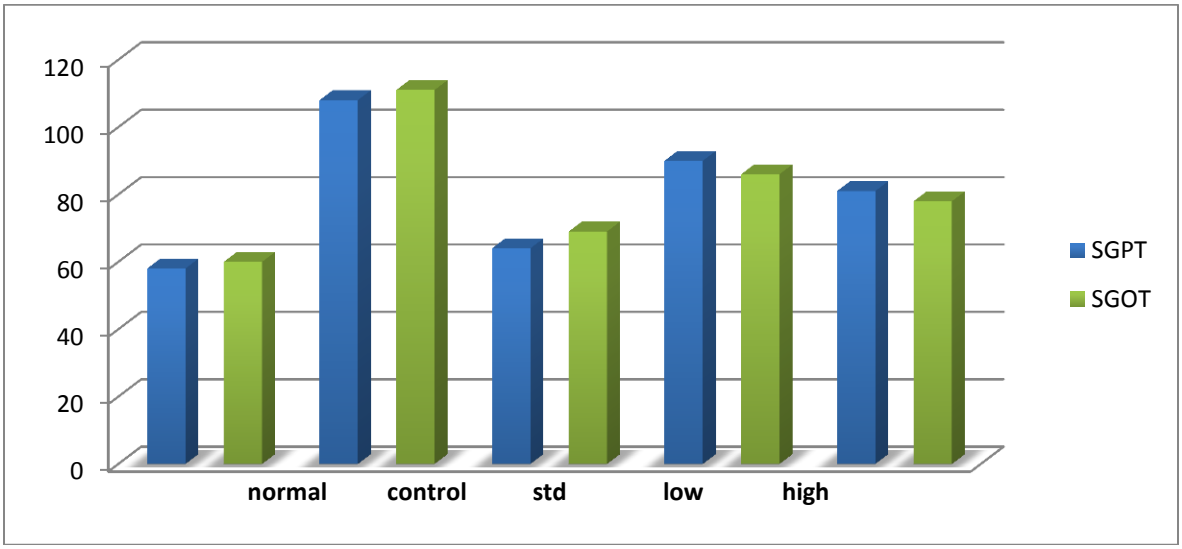

Fig. 3: Effect of ACAE on SGPT and SGOT level in STZ induced diabetic rats

RESULT AND DISCUSSION

Fasting blood glucose (FBG) level was within the range of 85-90mg/dl in all the groups at day 0. Treatment with STZ in normal saline (50mg/kg, i.p.) had increased the FBG level more than 200mg/dl after 48 h. Changes in FBG level in different groups after repeated dose of drug administration are tabulated in **Table No.2** and represented in **Fig.No.1**.Diabetic control group has showed significant increase in fasting blood glucose during the study period. Glibenclamide (5mg/kg) significantly ($p<0.01$) reduced FBG after repeated administration as compared to diabetic control group. Whereas treatment with ACAE at dose of 200 and 400mg/kg has significantly ($p<0.05$) decreased FBG as compared to diabetic control on 7^{th}, 14^{th}, 21^{st} and 30^{th} day.

Body weight of animals in all groups was recorded at 0, 7^{th} ,14^{th} and 21^{st} day. Highest change (decrease) in body weight during study period was found to be in diabetic control group. Glibenclamide and *Allamanda cathartica* aqueous extract treated groups showed increase in body weight as compared to diabetic control group **(Table 3, Fig 2)**.

In animals treated with Glibenclamide and *Allamanda cathartica* aqueous extract, SGPT and SGOT levels were decreased significantly ($p<0.001$, $p<0.01$ respectively) as compared to the diabetic control **(Table 4, Fig 3).** The levels of SGPT and SGOT in alloxan induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan. In this study, the aqueous extract of *A.cathartica* regulated the activity of SGPT and SGOT in liver of rats intoxicated with alloxan. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study. The restorations of SGPT and SGOT to their respective normal levels after treatment with both glibenclamide and aqueous extracts of *A.cathartica* further strengthen the antidiabegenic effect of these extract. More over SGPT and SGOT levels also act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver. Since the alloxan can also affect the liver by free radical mechanism

The anti-diabetic activity of ACAE could be due to the increased release of insulin from β 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 Strptozotocin 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 *Allamnda cathartica* aqueous extract has a potent anti diabetic effect which could be used for the management of diabetes effectively.

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