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Antidiabetic Activity of *Catharanthus Pusillus* (murr.) G. don (apocynaceae) Aerial Parts in Alloxan Induced Diabetic Wistar Albino Rats

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ABSTRACT

The present study deals with anti-diabetic activity of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) aerial parts in alloxan induced diabetic rats. Diabetes was induced by using alloxan monohydrate at the dose of 100 mg/kg body weight, to overnight fasted rats. Diabetes was confirmed by fasting blood glucose (FBG) levels, if it is more than 250 mg/dl considered as Diabetic. The methanol extracts of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) aerial parts (MECP) were administered orally to diabetic rats at the doses 250 and 500 mg/kg body weight. The effect of MECP on diabetes was compared with the standard drug Glibenclamide. MECP shows significant reduction in blood glucose level in dose dependent manner. The results obtained from this study proved that the plant *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) would be useful in the management of diabetes.

Keywords: *Catharanthus pusillus*, Alloxan, Diabetes mellitus, Fasting blood glucose level.

ARTICLE INFO

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1. Introduction

Diabetes mellitus is a chronic metabolic disease caused by a variable combination of insulin deficiency and insulin International Journal of Medicine and Pharmaceutical Research

resistance. The result is disordered utilization and storage of proximate nutrients (Carbohydrates, Proteins and Fats) and

reduced production of ATP (Satoskar, Bhandarkar, Nirmala, 2010). The incidence of diabetes is growing rapidly in worldwide. It is estimated more than 180 million people are afflicted with diabetes and prevalence is expected to more than double by the year 2030 (Richard Finkel, Luigi, Michelle, 2006). The major two forms of Diabetes type- I and type-II. Type-I is autoimmune disease also called as Insulin dependent diabetes mellitus, triggered by viral infection results in destruction of β -cells leads to insulin deficiency, ketoanemia and ketoacidosis. Other form is Type-II diabetes mellitus, which is also called non-insulin dependent diabetes mellitus; it is due to insulin resistance and obesity. Additional forms of diabetes mellitus include Gestational diabetes, which is associated with other endocrine disorder and exposure to drugs or chemical agents that are toxic to pancreas (George & Craig, 2008). Complications of diabetes mellitus classified into macro and micro vascular diseases which include neuropathy, nephropathy, cardiovascular and cerebrovascular diseases (Feldman, 1988). *Catharanthus pusillus* (Murr.) G. Don is annual erect herb also known as *Vinca pusillus*, belongs to Apocynaceae family. It can be grown up to one feet. It is widely distributed throughout India. In India, it is found in Andhra Pradesh, Delhi, Himachal Pradesh, Kerala, Orissa, Punjab, Tamil Nadu and West Bengal (Khare, 2009). Traditionally it is used as antitumor and antiulcer. It is also used to treat Paralysis, Epilepsy and Lumbago (Khare, 2009; Madhava Chetty et al., 2008; Pullaiah, 2002; Neelima et al., 2011), hysteria. The present research was aimed to investigate antidiabetic activity of *Catharanthus pusillus* (Murr.) G. Don aerial parts in alloxan induced diabetic rats.

2. Materials and Methods

Plant Collection and Authentication

The aerial parts of *Catharanthus pusillus* (Murr.) G. Don collected from forest of Thirupati, Chittoor dist. of Andhra Pradesh, India, in the month of December 2011 and authenticated by Dr. Madhava Chetty, Asst. Professor, Department of Botany, S.V University, Thirupati, Andhra Pradesh, India.

Preparation of Plant extract

The aerial parts the plant *Catharanthus pusillus* (Murr.) G. Don was dried at room temperature. The plant material was powdered by mechanically and sieved by passing through sieve no.40 mesh. The powdered material packed in the soxhlet apparatus and extracted with methanol for 48 hours. The extract was evaporated under vacuum dryer until the solvent is completely evaporated from the extract and the percentage yield was found to be 34% w/w.

Animals used

Wistar albino rats (150-200 g) were obtained from Sainath Enterprises, CPCSEA (769/CPCSEA/2010) approved breeder, Sainath Nagar, Uppal, Hyderabad, Andhra Pradesh. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard animal food and water was given *ad libitum*. Rats were acclimatized to laboratory conditions one week prior to initiation of experiments.

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Acute toxicity study

Acute toxicity studies of the methanol extracts of *Catharanthus pusillus* (Murr.) G. Don. aerial parts were carried out as per OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract did not produced mortality and morbidity even at 2000 mg/kg dose (OECD, 2002).

Induction of Diabetes Mellitus

For induction of diabetes, a single dose of Alloxan monohydrate at 100 mg/kg b.w, dissolved in normal saline (0.9 % w/v) was injected intraperitoneally, to the overnight fasted rats. After 72 hrs, blood glucose level was determined. Rats with fasting blood glucose levels more than 250 mg/dl were considered as diabetic rats and found with permanent diabetes were used for the study.

Statistical analysis

The data was expressed as mean \pm standard error mean (S.E.M) for six animals in each group. The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Tukey-Kramer multiple comparison test, the p values less than 0.05 were considered as significance.

Experimental Design

Animals were divided into five groups, each group containing six rats.

- Group 1: Served as normal control rats received Normal saline (0.9 % w/v).
- Group 2: Served as untreated (Disease control) rats received Alloxan monohydrate 100 mg/kg i.p single dose.
- Group 3: Diabetic rats treated with Standard drug Glibenclamide at the dose of 2.5 mg/kg.
- Group 4: Diabetic rats treated with methanol extract of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) (MECP) at the dose of 250 mg/kg, p.o, daily once for 10 days.
- Group 5: Diabetic rats treated with methanol extract of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) (MECP) at the dose of 500 mg/kg, p.o, daily once for 10 days.

Antidiabetic study

The effect of administration of extracts in alloxan induced diabetic rats were determined by measuring fasting blood glucose levels on 0, 4th, 7th and 10th day, as well as by estimating biochemical parameters. Blood samples were withdrawn from tail tip of rat for estimation of fasting blood glucose level and for estimation of biochemical parameters blood was collected from retro-orbital plexus under mild ether anaesthesia from overnight fasted rats at 10th day.

3. Results and Discussion

Results

Acute toxicity studies

Methanol extracts of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) is orally administered at the dose range of 100–2000 mg/kg b.w., to different groups. Each group comprised of ten rats. Mortality was observed after 14 days. MECP did not show any signs of side effects like body weights, behavioural changes. There was no mortality rate up to 2000 mg/kg.

Effects of MECP on fasting blood glucose level

Experimental studies reveal that the oral administration of MECP at the doses of 250 and 500 mg/kg exhibited hypoglycaemic effects in a dose dependent manner on 4th, 7th and 10th day of treatment. Glibinclamide produce a significant reduction in blood glucose level, the effect of MECP is compared with standard drug. The results are shown in table 2.

Effects of Methanolic extract of *Catharanthus pusillus* (Murr.) G.Don (Apocynaceae) on biochemical parameters (Table 3)

Biochemical parameters include Triglycerides (TG), Total Cholesterol (TC), High-density lipoproteins (HDL), Low density lipoproteins (LDL), Very low density lipoproteins (VLDL) and Serum Urea are estimated in serum which separated from the blood samples collected from rats on 10th day, at the end of the study. The effect of MECP on biochemical parameters were shown in table 3.

Discussion:

Methanolic extract of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) was selected for Antidiabetic activity in alloxan induced diabetic rats. Diabetes mellitus is complex and multifarious group of disorder characterised by hyperglycaemia that has reached epidemic proportions in the present century. Diabetes is leading to cause of morbidity and mortality among the population. Alloxan causes diabetes through its ability to destroy the insulin producing β -cell, which reduces the synthesis and the release of insulin (John Anne et al., 2004). Administration of alloxan increased serum glucose levels when compared to normal animals and also induced persistent diabetes mellitus in rats. My investigation reveals that Methanolic extract of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) decreases the serum glucose levels in alloxan induced diabetic rats. The concentration of lipids such as cholesterol, triglycerides, LDL cholesterol was

significantly increased and was as HDL cholesterol was decreased in diabetic rats than control rats. The impairment of insulin secretion results in enhanced metabolism of lipids from adipose tissue to plasma. A variety of de-arrangements in metabolic and regulatory mechanisms, due to insulin deficiency responsible for the observed accumulation of lipids (Rajalingam et al., 1993). Further it has been reported that diabetic rats treated with insulin shows normalised lipid levels (Pathak et al., 1981). Diabetic rats treated with methanolic extract of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) also shown normalised lipid levels. Thus the results indicate methanolic extract of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) also may possess insulin like actions by virtue of ability to lower the lipid levels. In the present study effect of methanolic, extract of *Catharanthus Pusillus* (Murr.) G. Don (Apocynaceae) on hyperglycaemia in alloxan induced diabetic rats. It was found that the fasting blood glucose levels (FBG) of the animals treated with *Catharanthus Pusillus* (Murr.) G.Don methanolic extract (Groups-IV & V) and the standard drug Glibinclamide (Group- III), significantly reduces when compared with diabetic control (Group- II) group. The FBG levels of all groups were observed on 0th, 4th, 7th and 10th day. The diabetic rats which are treated with *Catharanthus pusillus* (Murr.) G.Don and Glibinclamide showed a significant decrease in blood glucose level in 4th, 7th and 10th day. On 10th day FBG level of Group III decreases nearby to normal range. In this case of Group-IV & V animals FBG levels are significantly reduces, but it was less when compared to Group III. When compared with untreated group, the group of animals treated with *Catharanthus pusillus* (Murr.) G.Don showed significant reduction in blood glucose level at the doses of 250 and 500 mg/kg. This result gives an idea about that *Catharanthus pusillus* (Murr.) G.Don having significant hypoglycaemic effect.

Table 1: Effect of *Catharanthus pusillus* (Murr.) G.Don (Apocynaceae) on Body weights in Alloxan induced diabetic rats

Group	Treatment	Body weights (gm)			
		Initial	4 th Day	7 th Day	10 th Day
Group I	Normal control	181.50±3.75	183±2.78	185±2.80	189±2.86
Group II	Diabetic control	183.50±3.89	152±3.59	138.00±3.51	125.00±2.71
Group III	Alloxan+ Glibenclamide (5mg/kg)	186.60±3.84	183.00±3.64	176.50±3.15	162.50±2.25
Group IV	Alloxan+ MECP (250mg/kg)	185.40±3.39	178.00±2.15	171.50±2.89	162.60±2.24
Group V	Alloxan+ MECP (500mg/kg)	186.60±3.45	180.00±3.07	174.00±2.69	167.00±2.50

Table 2: Effect of Methanolic extract of *Catharanthus pusillus* (Murr.) G.Don (Apocynaceae) blood glucose levels in rats

Groups(n=6)	Fasting Glucose Levels (mg/dl)			
	0 Day	4 th Day	7 th Day	10 th Day
Group-I (Normal control)	91.46±3.80	93.82±2.92	93.32±1.73	94.29±3.44
Group-II (Diabetic control)	284.8 ±6	287.91±5.05	292.8±6.41	295±9.75
Group-III (Glibenclamide-5mg/kg)	286.48±7.32	206.25±8.06*	151.18±7.35*	102.13±7.20**
Group-IV (MECP 250mg/kg)	285.48±6.32	258.23±7.66*	186.85±8.8**	129.06±9**
Group-V (MECP -500mg/kg)	287.58±6.32	213.71±6.07***	189.26±4.52***	118.9±3.31***

Values are expressed as mean ±SEM of 6 rats in each group.*P<0.05, **P<0.001, ***P<0.001, as compared to diabetic control group.

Table 3: Effect of Methanolic extract of *Catharanthus pusillus* (Murr.) G.Don (Apocynaceae) on Lipid profile

Groups(n=6)	Biochemical Parameters					Serum Urea
	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	
Group-I (Normal control)	83.65±0.45***	95± 2.2	32.42±1.4	42.32±2.4	19.30±0.75***	30.09±1.09
Group-II (Diabetic control)	135.43±2.30	121.27± 2.2	26.02±1.2	89±6.4	52.42±4.23	62.93±0.6
Group-III (Glibenclamide-5 mg/kg)	85.14±0.85***	95.53±6.4*	31.53±1.6*	43.42±4.1*	23.85±0.43***	33.9± 1.4*
Group-IV (MECP 250 mg/kg)	105.27±0.63***	134.3± 3.4*	30.22±2.3	64.34±2.6*	32.54±0.34***	44.08±1.7*
Group-V (MECP -500 mg/kg)	93.10±0.58***	108.35±4.7*	29.47±3.2	48.26±3.2*	28.12±0.64***	37.33±0.7*

Values are expressed as mean ± SEM of 6 Rats in each group;

*p<0.01, **p<0.001, ***p< 000.1 as compared to diabetic control group

TG= Triglycerides, TC= Total Cholesterol, HDL= High Density Lipoprotein

LDL= Low Density Lipoprotein, VLDL= Very Low Density Lipoprotein

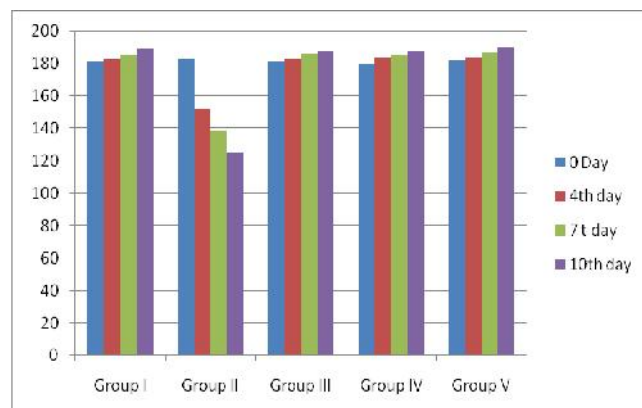


Figure 1: Effect of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) on Body weights in Alloxan induced diabetic rats

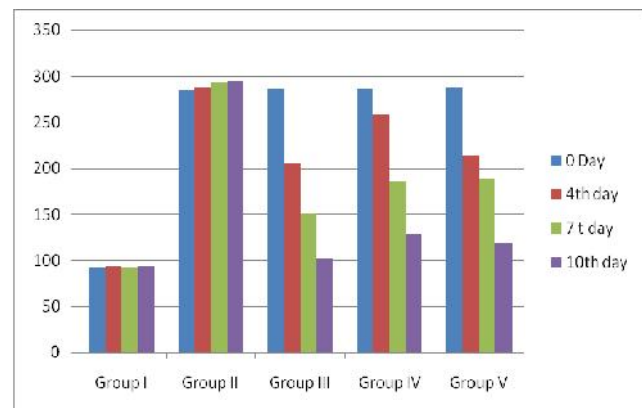


Figure 2: Effect of Methanolic extract of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) Aerial parts blood glucose levels in rats

In the present investigation the TG, TC, LDL and VLDL cholesterol was increased in diabetic control animals (untreated group) and it was decreased in 10 days treatment with *Catharanthus pusillus* (Murr.) G.Don as well as HDL cholesterol level was significantly increased. The total lipid profile in serum (TC, TG, HDL, LDL and VLDL) of the

alloxan induced animals treated with *Catharanthus pusillus* (Murr.) G.Don (250 and 500 mg/kg) was substantially improved, as compared to control diabetic group. These results give suggestion that *Catharanthus pusillus* (Murr.) G.Don may inhibit the cholesterol synthesis pathway and increased HDL/LDL ratio may be due to the activation of LDL receptors in hepatocyte, which is responsible for taken up LDL into the liver, and reduce the serum the serum LDL levels (Rang et al., 1999). The preliminary phytochemical screening of methanolic extract of *Catharanthus pusillus* (Murr.) G.Don (Apocynaceae) indicates that the presence of Alkaloids, Carbohydrates, Saponins and Flavanoids. These compounds are responsible for the therapeutic effect. May be the presence these constituents in the Methanolic extracts responsible for hypoglycaemic activity

4. Conclusion

It is concluded the methanol extract of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) possesses significant property and may prove to be effective for the treatment of diabetes mellitus. Further studies on pharmacological and biochemical investigations will clearly elucidate the mechanism of action to develop as a potent antidiabetic drug

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