



STUDY OF ANTI HYPERGLYCEMIC EFFECT OF *CATHARANTHUS ROSEUS* IN ALLOXAN INDUCED DIABETIC RATS

M. JAYANTHI¹, N. SOWBALA¹, G. RAJALAKSHMI¹, U. KANAGAVALLI¹ AND V. SIVAKUMAR^{2*}

P.G and Research Department of Biochemistry, Adhiparasakthi College of Arts and Science, Kalavai, Vellore District - 632 506, Tamilnadu, India, ²Department of Pharmacy, Sri Lakshmi Narasimha College of Pharmacy, Palluru-517 132, Andhra Pradesh, India
Email: sivakumarv2k1@gmail.com

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ABSTRACT

The present work was carryout to study the effect of daily oral administration of *Catharanthus roseus* (CR) leaf dichloromethane: methanol (1:1) extracts (500 mg/ body weight) for 20 days on blood glucose and hepatic enzymes in normal and Alloxan induced diabetic rats. A significant ($P<0.05$) increased body weight and decreased blood glucose, urea, cholesterol levels of the test animals shows that the extract exhibited anti hyperglycemic activity and increased in protein and glycogen ($P<0.01$) where observed in diabetic rats treatment with *Catharanthus roseus* leaf dichloromethane methanol extract when compared to diabetic rats. The activity of the hepatic enzymes such as hexokinase was significantly ($P<0.01$) increased and glucose 6-phosphatase and fructose 1, 6- bisphosphatase were significantly ($P<0.05$) decreased by the administration of *Catharanthus roseus* leaf in diabetic rats when compared to normal rats.

Keywords: *Catharanthus roseus*, Alloxan, Diabetes mellitus, Hepatic enzymes

INTRODUCTION

Diabetes mellitus is one of the commonest chronic illnesses to human beings. It is characterized by deleterious hyperglycemia is one of the leading disease in the world¹. The world health organization (WHO 1999) estimates that by the year 2030, the number of people with diabetes will have reached 370 million². There is a high level of treatment failures and unpleasant side effects associated with oral anti diabetic drugs generating an urgent need and desire for alternative treatment by the use of plant based products are becoming popular in the treatment and management of diabetes³.

Catharanthus roseus (Vinca rosea) a traditionally used medicinal plant, belongs to the family Apocynaceae, is an erected procumbent herb or under shrub containing latex. It is widely growing to 1m tall at subtropical area. The leaves are oval to oblong, 2.5 – 9.0cm long and 1.0- 3.5 cm broad, glossy green, hairless, with a pale midrib and a short petiole 1.0- 1.8 cm long; they are arranged in opposite pairs. The flowers are white to dark pink with a darker red centre, with a basal tube 2.5 – 3.0 cm long and a corolla 2.0 – 5.0 cm diameter with five petals like lobes. The fruits are a pair of follicles 2.0- 4.0 cm long and 3 mm broad⁴. This plant have possesses known antibacterial, antimicrobial, antifungal, antioxidant, anticancer and antiviral activates⁵. The aim of the present study is an attempt to assess the antihyperglycemic activity of dichloromethane: methanol extract (1:1) of *Catharanthus roseus* in normal and Alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant materials

Catharanthus roseus plant leaf was collected from Adhiparasakthi Agricultural College Medicinal Park in Kalavai. The plant was identified at the Herbarium of Botany Directorate in Adhiparasakthi Agricultural College. A voucher specimen (No: Cr09) was deposited in the Botany Department of Adhiparasakthi Agricultural College, Kalavai, Tamilnadu, India.

Animals

Healthy adult cross breed male Wistar albino rats (weighing 150 – 220g) were used throughout the experiment. Animals were maintained at $22 \pm 20^\circ\text{C}$ with 45 – 55% relative humidity, 12 hours light and dark cycle. They were housed in well-ventilated

polyurethane cages and had free access to tap water and laboratory pellet feed.

Preparation of plant extract

Dried leaf powder of *Catharanthus roseus* was allowed to pass through ss sieve (20 meshes) and then extracted in soxhlet extraction apparatus with dichloromethane: methanol extract (1:1). The solvent was removed under vacuum to get solid mass. The extracted dry powder was dissolved in physiological saline solution and given orally to diabetic group and normal group rats at a concentration of 500mg per Kg of body weight once daily up to 20 days.

Experimental induction of diabetic mellitus

The experimental animal in this model is the male, adult Wistar albino rats, weighing 150–220g. After a 48-hour fast, the rats were weighed and a solution of 2% alloxan diluted in saline (0.9%) corresponding to 80 mg of alloxan per kg of body weight was administered intraperitoneally in a single dose. Food and water were given to the rats after 30 minutes of drug administration^{6,7}.

Experimental designs

In the experiment a total of 24 rats (12 diabetic surviving rats, 12 normal rats) were used. After one month, the rats were divided into four groups each group consisting of six rats.

Group I - Normal rats.

Group II - Normal rats were given CR dichloromethane methanol extract 500 mg /Kg body weight daily up to 20 days.

Group III - Diabetic rats.

Group IV - Diabetic rats were given CR dichloromethane methanol extract 500 mg /Kg body weight daily up to 20 days.

Sample collection

At the end of 20th days the animals were deprived of food overnight and sacrificed by decapitation. Fasting blood sample was collected in fresh vials containing sodium fluoride and potassium oxalate (anticoagulant agent) for the estimation of blood glucose, blood urea, cholesterol, and blood serum was prepared for the estimation of protein. Liver is dissected out and washed in ice-cold saline immediately.

Evaluation of effect on biochemical variables

Fasting blood glucose⁸, protein⁹ urea¹⁰ and cholesterol¹¹ were estimated. The liver supernatant was extracted and used for the estimation of liver glycogen¹² and assay of Hexokinase¹³, Fructose - 1, 6- bisphosphatase¹⁴, Glucose -6- phosphatase¹⁵.

Statistical analysis

Statistical treatment applied is ANOVA under two way classification, changes were considered significant if the P-Value was <0.01 and <0.05. The values are expressed as mean \pm SD.

Table 1: Levels of blood glucose, blood urea, cholesterol and protein in normal, *Catharanthus roseus* treated control, diabetic and *Catharanthus roseus* treated diabetic rats

Groups	Blood glucose (mg/dl)	Blood urea (mg/dl)	Cholesterol (mg/dl)	Protein (g/dl)
Normal	79.18 \pm 5.2*	25.9 \pm 1.9*	90.6 \pm 3.3*	7.53 \pm 0.1**
<i>Catharanthus roseus</i> treated control	74.12 \pm 5.6*	24.6 \pm 2.9*	98.2 \pm 6.9*	8.02 \pm 0.3**
Diabetes	241.62 \pm 2.3*	63.5 \pm 0.5*	186.8 \pm 8.0*	6.70 \pm 0.5**
<i>Catharanthus roseus</i> treated diabetes	110.08 \pm 8.6*	38.4 \pm 3.8*	134.8 \pm 1.4*	7.2 \pm 0.3**

Values are expressed as mean \pm SD for six animals in each group. *Level of significance $p < 0.05$, **Level of significance $p < 0.01$

The level of total protein, decreased significantly in diabetic rats because of the insufficient of insulin leads to increased protein degradation and decreased protein synthesis and the level restored after 20 days treatment of *Catharanthus roseus* plant leaf. The elevated level of blood urea, and cholesterol observed in diabetic rats is found to be corrected to near normal ($P < 0.05$) in the dichloromethane methanol *Catharanthus roseus* plant leaf extract treatment. Table 2 shows the level of liver glycogen and animal body

RESULTS AND DISCUSSION

The present study was conducted to evaluate the antihyperglycemic activity of *Catharanthus roseus* plant leaf dichloromethane methanol extract in alloxan induced diabetes rats.

Diabetic rats treated with crude dichloromethane methanol extract at oral dose of 500 mg/kg for 20 days Table -1 shows exhibited significant reduction in blood glucose in comparison to untreated diabetic rats. Blood glucose levels of diabetic and treated animals wear 241.62 \pm 2.3, and 110.08 \pm 8.6mg/dl respectively ($P < 0.05$).

weight. In diabetic group animals sharp decline in body weight¹⁶ and liver glycogen level was compared to normal groups¹⁷, after treatment of *Catharanthus roseus* plant leaf improved in the body weight and stored glycogen level. The restored liver glycogen level may be considered as the best marker for assessing antihyperglycemic activity of *Catharanthus roseus* plant leaf extract treatment.

Table 2: Levels of Body weight and Liver glycogen in normal, *Catharanthus roseus* treated control, diabetic and *Catharanthus roseus* treated diabetic rats.

Groups	Body weight(g)		Glycogen (mg/g wet tissue)
	Initial (g)	Final (g)	
Normal	182.03 \pm 14.1	201.5 \pm 4.2	44.8 \pm 2.4**
<i>Catharanthus roseus</i> treated control	187.07 \pm 10.6	219.6 \pm 4.5	52.2 \pm 2.3**
Diabetes	189.06 \pm 03.2	152.3 \pm 6.2	22.5 \pm 0.6**
<i>Catharanthus roseus</i> treated diabetes	194.03 \pm 04.3	205.4 \pm 4.6	42.2 \pm 2.3**

Values are expressed as mean \pm SD for six animals in each group. *Level of significance $p < 0.05$ **Level of significance $p < 0.01$

The changes in the activities of hepatic enzyme hexokinase, glucose 6-phosphatase and fructose 1, 6- bisphosphatase of normal, control, diabetic and treatment groups are shows in table 3. In experimental diabetic rats, enzymes of glucose metabolism are markedly altered leads to pathogenesis of diabetic complications. The activity of hexokinase enzyme decreased in the alloxan induced diabetic rats. *Catharanthus roseus* dichloromethane methanol extract administration to alloxan induced rats resulted in an increased activity ($P < 0.01$) of liver hexokinase, the increased activity to leads

increased glycolysis and utilization of glucose for enzyme production¹⁸.

The activity of the glucose 6-phosphatase and fructose 1, 6- bisphosphatase were found to be increased in diabetic rats¹⁹, (table 3) in the present study glucose 6-phosphatase and fructose 1, 6- bisphosphatase activity was brought to near normal ($P < 0.05$) on treatment with *Catharanthus roseus* dichloromethane methanol extract at 500 mg/ body weight for 20 days.

Table 3: Activities of hexokinase, fructose 1, 6 - bisphosphatase, glucose - 6 - phosphatase in liver of normal, *Catharanthus roseus* treated control, diabetic and *Catharanthus roseus* treated diabetic rats.

Groups	Hexokinase ^a	Fructose 1, 6 -bisphosphatase ^b	Glucose - 6 - phosphatase ^b
Normal	187.2 \pm 7.5*	489.2 \pm 4.6*	1043.2 \pm 9.6*
<i>Catharanthus roseus</i> treated control	184.6 \pm 7.6*	479.9 \pm 5.5*	990.26 \pm 1.3*
Diabetes	130.3 \pm 1.5*	770.1 \pm 7.5*	1260.58 \pm 2.7*
<i>Catharanthus roseus</i> treated diabetes	171.1 \pm 8.4**	553.8 \pm 1.0*	1163.62 \pm 6.4*

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

*Level of significance $p < 0.05$, **Level of significance $p < 0.01$ a. μ - moles of glucose - 6 - phosphate formed/h/mg protein, b. n moles of phosphorous liberated/h/mg protein.

CONCLUSION

In conclusion, the *Catharanthus roseus* plant leaf dichloromethane methanol extract was found to exhibit a significant anti hyperglycemic activity in alloxan induced diabetic rats. The further works was to isolate the active principal phytoconstituents and assess the bioactivity of the *Catharanthus roseus* plant extract.

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