

Antihyperglycemic and Antihyperlipidaemic effect of *Polygala rosmarinifolia* Wright & Arn on alloxan induced diabetic rats

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ABSTRACT

The ethanol extract of *Polygala rosmarinifolia* whole plant (Family: Polygalaceae) was investigated for its antihyperglycemic and antihyperlipidaemic effect in Wistar Albino rats. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg, i.p). The ethanol extracts of *Polygala rosmarinifolia* at a dose of 100 and 200mg/kg of body weight were administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extract of *Polygala rosmarinifolia* whole plant extract on blood glucose, serum insulin, urea, creatinine, glycosylated haemoglobin, serum lipid profile [total cholesterol (TC), triglycerides (TG), low density lipoprotein – cholesterol (LDL-C), very low density lipoprotein–cholesterol (VLDL-C), high density lipoprotein – cholesterol (HDL-C) and phospholipid (PL)] serum protein, albumin, globulin, serum enzymes [serum glutamate pyruvate transaminases (SGPT), serum glutamate oxaloacetate transaminases (SGOT), and alkaline phosphatase (ALP)], were measured in the diabetic rats. The ethanol extract of *Polygala rosmarinifolia* whole plant elicited significant reductions of blood glucose ($P<0.05$), lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C. The extracts also caused significant increase in serum insulin ($P<0.05$) in the diabetic rats. From the above results, it is concluded that ethanol extract of *Polygala rosmarinifolia* possesses significant antihyperglycemic and antihyperlipidaemic effects in alloxan induced diabetic rats.

INTRODUCTION

Diabetes mellitus (DM), a leading non communicable disease with multiple aetiologies, affects more than 100 million people Worldwide and is consider as one of the leading causes of death in the world (Zimmet, 1999). The World Health Organization (WHO) reported that 300 million peoples would suffer from diabetes mellitus by the year 2025 (Pradeepa and Mohan, 2002). Diabetes mellitus is characterized by an increased concentration of blood glucose due to derangement in carbohydrates metabolism and defective secretion of insulin. There metabolic disturbances result in acute and long term diabetic complications, which are responsible for premature death and disability (Aravind *et al.*, 2002).

Diabetes mellitus is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rats, defects in reactive oxygen species scavenging enzymes and high oxidative stress induced damage to pancreatic beta cells (Ugochukwa and Babady, 2003; Scoppoia *et al.*, 2001; Owu *et al.*, 2006; Kesavalu *et al.*, 2000). Diabetes mellitus is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into account (Trivedi *et al.*, 2004; Sharma *et al.*, 2010). In traditional practice, medicinal plants are used in many countries to control DM. The National Center for Complementary and Alternative Medicine, established in 1998 by the United States Government where development of herbal medicine is one of the important subjects of study (Yoon *et al.*, 2004). WHO has recommended evaluation of plants effective in different diseases. Many Indian Medicinal plants have been found to be useful in successfully managing diabetes and from

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some of them active principles have been isolated (Shukla *et al.*, 2000). Thus, it will be useful to look for new and if possible more efficacious drugs and the vast reserves of phytotherapy may be an ideal target. *Polygala* was traditionally used by Americans to treat snake bites and as an expectorant to treat cough and bronchitis. *Polygala* is considered as a powerful tonic than can help to develop the mind and aid in creative thinking. To our knowledge no report on the effect of *Polygala rosmarinifolia* whole plant on experimental antidiabetic. This study was therefore undertaken to evaluate the effects an ethanol extract of whole plant of *Polygala rosmarinifolia* on antihyperglycemic and anti hyperlipidemic activity in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant Material

The whole plant of *Polygala rosmarinifolia* were freshly collected from the well grown healthy plants inhabiting the natural forests of Maruthamalai, Coimbatore, Tamil Nadu. The plant were identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for phytochemical screening and antidiabetic studies

The *Polygala rosmarinifolia* whole plant were shade dried at room temperature and the dried whole plant were powdered in a Wiley mill. Hundred grams of powdered *Polygala rosmarinifolia* whole plant was packed in a Soxhlet apparatus and extracted with ethanol. The extract were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures (Brinda *et al.*, 1981; Lala, 1983). The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

Animals

Normal healthy male Wistar albino rats (180- 240g) were housed under standard environmental conditions at temperature (25±2° C) and light and dark (12: 12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If

mortality was not observed, the procedure was repeated for higher doses such as 50,100, and 2000 mg/kg body weight.

Induction of Diabetes in Experimental animal

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg) (Nagappa *et al.*, 2003). Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200-260 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Experimental Design

In the present investigation, a total of 30 rats (24 diabetic surviving rats and 6 normal rats) were taken and divided into five groups of 6 rats each.

Group I: Normal untreated rats

Group II: Diabetic control rats

Group III: Diabetic rats given ethanol extract of *Polygala rosmarinifolia* whole plant (100mg/kg body weight)

Group IV: Diabetic rats given ethanol extract of *Polygala rosmarinifolia* whole plant (200mg/kg body weight)

Group V: Diabetic rats given standard drug glibenclamide (600µg/kg body weight).

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes.

Estimation of insulin, glucose, urea, creatinine and glycosylated haemoglobin

Serum glucose was measured by the O-toluidine method (Sasaki *et al.*, 1972). Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit (Anderson *et al.*, 1993). Urea estimation was carried out by the method of Varley (Varley, 1976); serum creatinine was estimated by the method of (Owen *et al.*, 1954). Glycosylated haemoglobin (HBA₁C) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan (1985).

Estimation of protein, albumin, globulin, SGPT, SGOT, ALP

Serum protein (Lowry *et al.*, 1951) and serum albumins was determined by quantitative colorimetrically method by using bromocresol green. The total protein minus the albumin gives the globulin, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by utilizing the method of Reitman and Frankel (1957). Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong (1934).

Estimation of lipids and lipoprotein

Serum total cholesterol (TC) (Parekh and Jung, 1970), total triglycerides (TG) (Rice, 1970), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol

Table. 1: Effect of ethanol extract of *Polygala rosmarinifolia* whole plant on the serum insulin, glucose, urea, creatinine and HBA₁C level of normal, diabetic induced and drug treated adult albino rats.

Groups	Glucose (mg/dl)	Insulin (MIu/ml)	Urea (mg/dl)	Creatinine (mg/dl)	HB A ₁ C (%)
I	83.16±2.11	14.27±0.74	18.16±0.94	0.69±0.03	3.91±0.11
II	231.84±18.43**	5.21±0.24**	36.69±1.14**	0.93±0.04*	8.59±0.14**
III	168.14±1.94 ^a	8.36±0.16 ^a	28.31±0.87 ^a	0.88±0.06	6.39±0.13 ^a
IV	114.34±2.08 ^{aa}	11.96±0.26 ^a	14.54±0.26 ^a	0.73±0.08	4.99±0.24 ^a
V	94.66±1.84 ^{aaa}	13.63±0.18 ^{aaa}	16.24±0.39 ^a	0.77±0.06	4.07±0.14 ^a

Each Value is SEM ± 6 individual observations * $P < 0.05$; ** $P < 0.01$ Compared normal control vs -Diabetic rats : a - $P < 0.05$; aa - $P < 0.01$ Compared - Diabetic rats vs drug treated.

Table. 2: Effect of ethanol extract of *Polygala rosmarinifolia* whole plant on the serum protein, albumin, globulin, SGOT, SGPT and ALP level of normal, diabetic induced and drug treated adult albino rats.

Parameter	Protein (g/dl)	Albumin (g/dl)	Globulin(g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group I	8.14±0.17	4.50±0.18	3.64±0.13	18.31±0.61	21.66±0.34	120.16±4.11
Group II	5.27±0.11*	2.32±0.13*	2.95±0.16*	29.11±0.36**	34.16±0.71*	191.20±3.92**
Group III	6.34±0.08	3.48±0.06	2.86±0.14	22.30±0.28	30.46±0.36	158.10±1.48
Group IV	7.56±0.11	4.16±0.10	3.40±0.11	16.27±0.31	26.27±0.14	143.12±2.19
Group V	8.01±0.31	4.23±0.14	3.78±0.25	18.16±0.23	24.30±0.14	124.66±2.74

Each Value is SEM ± 6 individual observations * $P < 0.05$; ** $P < 0.01$ Compared normal control vs -Diabetic rats.

Table. 3: Effect of *P. rosmarinifolia* extract on serum lipid profile in the normal, diabetic and drug treated rats.

Groups	TC (mg/dl)	TG (mg/dl)	HDL – C (mg/dl)	LDL – C (mg/dl)	VLDL – C (mg/dl)	PL (mg/dl)
I	103.11±2.56	92.66±1.87	21.11±1.32	63.47±2.13	18.53±1.15	159.76±2.67
II	214.19±1.84**	178.26±2.19**	06.29±2.36**	92.25±2.56*	35.65±1.45	258.62±3.46
III	164.36±1.20 ^a	121.12±1.64 ^a	18.64±1.08 ^a	80.63±1.26 ^a	26.48±1.34	188.54±2.42
IV	108.26±1.33 ^a	84.64±1.72 ^a	30.16±1.30	61.18±1.32 ^a	16.92±1.14	164.35±2.88
V	116.84±1.63 ^a	91.55±1.14 ^a	25.34±1.11 ^a	73.19±1.59 ^a	18.31±1.08	171.98±2.57

Each Value is SEM ± 6 individual observations * $P < 0.05$; ** $P < 0.01$ Compared normal control vs -Diabetic rats a - $P < 0.05$; Compared -Diabetic rats vs drug treated.

(VLDL- C) (Friedwald *et al.*, 1972), high density lipoprotein cholesterol (HDL-C) (Warnick *et al.*, 1985) and phospholipids (Takayama *et al.*, 1977) were analyzed.

Statistical Analysis

The data were analyzed using student's t-test statistical methods. For the statistical tests a p values of less than 0.01 and 0.05 was taken as significant.

RESULT AND DISCUSSION

The phytochemical screening of ethanol extract of *Polygala rosmarinifolia* whole plant revealed the presence of alkaloid, catechins, coumarin, tannin, saponin, steroid, flavonoid, phenol, sugar, glycoside and xanthoprotein. Acute toxicity study revealed the non-toxic nature of the ethanol extract of *P. rosmarinifolia* whole plant. Table 1 shows the levels of blood glucose, serum insulin, urea, creatinine and glycosylated haemoglobin of normal, diabetic and drug treated rats. The alloxan induced diabetic rats elicited significant rise in blood glucose from 83.16 to 231.84 mg/dl ($p < 0.01$) and a significant decrease in serum insulin level from 14.27 to 5.21 ($p < 0.01$). On the contrary, diabetic rats treated with ethanol extract of *Polygala rosmarinifolia* whole plant exhibited decrease in blood glucose and increase in serum insulin significantly at a dose of 100 mg/kg and 200mg/kg body weight. It was observed that ethanol extract of *Polygala rosmarinifolia* reversed these effects in diabetic animals. It has been reported that

using medicinal plant extract to treat alloxan induced diabetic rats results in activation of β -cells and insulinogenic effects (Shunmugasundaram *et al.*, 2011). *Polygala rosmarinifolia* may also have brought about hypoglycemic action through stimulation of surviving of β -cells of islets of langerhans to release more insulin. This was clearly evidenced by the increased levels of serum insulin in diabetic rats treated with *Polygala rosmarinifolia*. Since the percentage fall in blood glucose levels was different in models with varying intensity of hyperglycemia, it implies that the antihyperglycemic effect of that plant is dependent on the dosage of diabetogenic agent, which in turn leads to β -cells destruction (Grover *et al.*, 2000). Earlier many plants have been studied for their hypoglycemic and insulin release stimulatory effects. (Pattabiraman and Muthukumar, 2011; Maruthupandian and Mohan, 2011; Shunmugasundaram *et al.*, 2011; Kala *et al.*, 2012a, 2012b; Shajeela *et al.*, 2012).

A significant elevation in serum constituents, urea and creatinine were observed in alloxan induced diabetic rats (Group II), when compared to control rats. The ethanol extract of *P. rosmarinifolia* whole plant was administered orally (100mg/kg body weight-Group III 200mg/kg body weight-Group IV) to rats for 14 days, reversed to urea and creatinine level to near normal. The administration of glibenclamide (Group V) also decreased the levels of urea and creatinine to some extent. Urea is the main end product of protein metabolism. Amino acid deamination takes place in the liver, which is also the site of urea cycle, where

ammonia is converted into urea and excreted through urine. Urea varies directly with protein intake and inversely with the rate of excretion. Some of the urea is bound to haemoglobin so its concentration in red blood cells is greatly than in the plasma. Renal diseases which diminish the glomerular filtration lead to urea retention and decrease in urea is seen in severe liver disease with destruction of cells leading to impairment of the urea cycle (Ranjna, 1999). Significant ($P < 0.05$) decrease in serum urea observed with diabetic rats treated with *P. rosmarinifolia* (200mg/kg body weight) may not be as a result of liver damage or abnormal functional kidney. Creatinine is a waste product formed in muscle by creatinine metabolism. Creatinine is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle. Its retention in the blood is evidence of kidney impairment. The present results show that, the treatment with ethanol extract of *P. rosmarinifolia* whole plant was effective in preventing alloxan induced increase in serum creatinine level when compared in the control.

Glycosylated haemoglobin has been found to be increased over a long period of time in diabetes. During diabetes, the excess of glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin (Alyassin *et al.*, 1981). The rate of glycation is proportional to the concentration of blood glucose. In present study, alloxan induced diabetic rats showed significant increase ($P < 0.01$) glycosylated haemoglobin (HBA_{1C}) level compared with normal rats. The ethanol extract of *P. rosmarinifolia* whole plant treated rats showed a significant decrease ($P < 0.05$) in the content of glycosylated haemoglobin that could be due to an improvement in glycemic status.

The levels of serum protein, albumin and globulin of control, alloxan induced diabetic rats and drug treated rats were presented in Table 2. A significant reduction in serum protein, albumin and globulin were observed in alloxan induced diabetic rats (Group II) when compared to control (Group I) and glibenclamide treated rats (Group V). This is in agreement with hypoalbuminemia observed in diabetes (Porte and Hatler, 1981). On the other hand, in the *P. rosmarinifolia* extract treated diabetic rats protein metabolism never deviated from normal range. Hypoalbuminemia is a common problem in diabetic animals and is generally attributed in the presence of nephropathy. An overall reduction in serum total protein in diabetic animals and consequents albumin were observed in the present study. The reversal of these changes by ethanol extract of *P. rosmarinifolia* therapy proved that insulin deficiency has been grossly corrected. Table 2 summarized the effect of alloxan on the activity of the hepatic marker enzymes in serum. There is an increase in transaminase activities in the serum of diabetic animals. The increased levels of transaminases, which are active in the absence of insulin because of increased availability of amine acids in diabetes, are responsible for the increased glucogenesis and ketogenesis observed in diabetes (Sivajothi *et al.*, 2008). There is an improvement noticed in the levels of SGOT, SGPT and ALP are as a consequence of improvement in the carbohydrate, fat and protein metabolism due to the administration of ethanol extract of

P. rosmarinifolia. The restoration of SGOT, SGPT and ALP to their normal levels may be due to the presence of flavonoids in the ethanol extract of *P. rosmarinifolia*, which are reported to be hepatoprotective agents (Ahmed *et al.*, 2000).

The levels of serum lipid profile, total cholesterol (TC), triglycerides (TG), LDL-C, VLDL-C and HDL-C in control, diabetic induced and drug treated rats were presented in Table 3. Alloxan induced rats showed significant increase in serum lipid profiles except HDL-C when compared with normal rats. The glibenclamide (Group V) and ethanol extract of *P. rosmarinifolia* (Group III and IV) treated rats showed a significant decrease in the content of lipid profiles when compared with diabetic induced rats. Similarly HDL-C level decreased in alloxan induced diabetic rats when compared to normal rats. On administration of ethanol extract of *P. rosmarinifolia* and glibenclamide to the diabetic rats, HDL-C level was found to be restored to normal. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency are responsible for the observed accumulation of lipids (Rajalingam *et al.*, 1993). The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. Further it has been reported that diabetic rats treated with insulin shows normalized lipid levels (Pathak *et al.*, 1981). Thus the results indicate that *P. rosmarinifolia* shows insulin-like action by virtue of its lipid lower levels. Phospholipids were increased in alloxan induced diabetic rats. Phospholipids are present in cell membrane and makeup vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with polar plasma environment and non-polar lipoprotein core (Cohn and Roth, 1996). Increased phospholipids levels in tissues were reported by Venkateswaran *et al.*, (2002) and Pari and Satheesh (2004) in alloxan diabetic rats. Administration of ethanol extract of *P. rosmarinifolia* whole plant and glibenclamide decreased the levels of phospholipids.

It is concluded that, medicinal plants have been reported to possess antihyperglycemic activity. *P. rosmarinifolia* whole plant is gaining much importance in diabetic control, since the phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, glycosides, steroids, tannins, saponin and phenols. Several authors reported that flavonoids, steroids, terpenoids, phenolic acids are known to be bioactive antidiabetic principles (Oliver 1980). Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secretagogues (Chakravarthy *et al.*, 1980). Saponin reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intraluminal physicochemical reaction. Hence, it has been reported to have hypocholesterotemic effect and thus may aid lessening metabolic burden that would have been placed in the liver (Rajan *et al.*, 2012). In the present study, the phytochemical analysis of ethanol extract of *P. rosmarinifolia* clearly pointed out the presence of above said active phytochemicals. It denotes that the antidiabetic effect of ethanol extract of *P. rosmarinifolia* may be due to the presence of more than one antihyperglycemic principle and their synergistic effects.

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