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Antidiabetic and Antihyperlipidaemic activity of *Polygala chinensis* L whole plant in alloxan induced diabetic rats

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ABSTRACT

The ethanol extract of *Polygala chinensis* whole plant (Family: Polygalaceae) was investigated for its antidiabetic 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 chinensis* 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 chinensis* whole plant extract on blood glucose, serum insulin, urea, creatinine, glycosylated haemoglobin, serum lipid profile [total cholesterol (TR), 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), and serum glutamate oxaloacetate transaminases (SGOT), and alkaline phosphatase (ALP)], were measured in the diabetic rats. The ethanol extract of *Polygala chinensis* 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 chinensis* possesses significant antidiabetic and antihyperlipidaemic effects in alloxan induced diabetic rats.

Keywords: Antihyperlipidaemic, Antidiabetic, *P. chinensis,* Alloxan and Glibenclamide.

1. INTRODUCTION

According to World Health Organization (WHO) projections, the prevalence of diabetes is likely to increase by 35% by the year 2025 [1]. The prevalence of diabetes is estimated to reach 330 million by the year 2025, according to International Diabetes Federation, with the greatest potential increase being in Africa and Asia. This numerical increase will occur in developing countries. Thus by the year 2025 over 75% of people with diabetes will reside in developing countries, as compared to 62% in 1995 [2, 3]. Diabetes mellitus is a chronic metabolic disorder in the endocrine system with multiple etiology, is characterized by chronic hyperglycemia together with disturbances in carbohydrate, protein and fat metabolism results from a decrease in circulating concentration of insulin (insulin deficiency), a decrease in the response of peripheral tissues to insulin (insulin resistance) or both [4]. It is becoming the third "killer" of mankind after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality [5]. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various orgens [6].

Recently the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulfonylureas, d-phenylalanine and α-glucosidase inhibitors in addition to insulin. However, due to unwanted side effects in the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes [6]. Hence plants have been suggested as a rich, as yet unexplored source of potentially useful antidiabetic drugs. Many traditional plants treatment for diabetes are used throughout the world.

According to the World Health Organization (WHO) estimates that 80% of people in developing countries depend on traditional medicine for their health needs, and 85% of traditional medicine involves the use of plant extracts. In other words, about 4 billion people in the world rely on plants as source of drugs. Plant drugs and herbal formulations are frequently

considered to be less toxic and free from side effects than synthetic one [8].The antihyperglycemic effects of these plants are for their ability to restore the function of pancreatic tissues by increasing insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes.

This has led researchers to continue their search for the "miracle drug" for treatment of diabetes from plants. *Polygala chinensis* L. belongs to Polygalaceae family. It is commonly known as "Siriyanangai". Genus *Polygala* was traditionally used by Americans to treat snake bites [9] and as an expectorant to treat cough and bronchitis. *Polygala* is considered as a powerful tonic herb [10] that can help to develop the mind and aid to creative thinking. Taking into the consideration of the medicinal importance of *Polygala,* the present study was conducted to investigate the antidiabetic and antihyperlipidaemic activities of ethanol extract of whole plant of *Polygala chinensis* in alloxan induced diabetic rats.

2. MATERIALS AND METHODS

2.1. Plant Material

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

2.2. Preparation of plant extract for phytochemical screening and antidiabetic studies

The *P. chinensis* whole plant was shade dried at room temperature and the dried whole plant was powdered in a Wiley mill. Hundred grams of powdered *P. chinensis* whole plant was packed in a Soxhlet apparatus and extracted with ethanol. The extracts were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures [11, 12]. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

2.3. Animals

Normal healthy male Wistar Albino rats (180- 240g) were housed under standard environmental conditions at temperature (25±2º 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*.

2.4. 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 [13]. 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.

2.5. Induction of Diabetes in Experimental animal

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg) [14]. 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.

2.6. 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 *P. chinensis* whole plant (100mg/kg body weight)

Group IV: Diabetic rats given ethanol extract of *P. chinensis* 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.

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

Serum glucose was measured by the O-toluidine method [15]. Insulin level was assayed by Enzyme Linked Immuno Sorbant Assay (ELISA) kit [16]. Urea estimation was carried out by the

method of Varley [17]; serum creatinine was estimated by the method of Owen *et al* [18]. Glycosylated haemoglobin (HBA_1C) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan [19].

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

Serum protein [20] 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 [21]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong [22].

2.6.3. Estimation of lipids and lipoprotein

Serum total cholesterol (TC) [23], total triglycerides (TG) [24], low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL- C) [25], high density lipoprotein cholesterol (HDL-C) [26] and phospholipids [27] were analyzed.

2.7. 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.

3. RESULTS AND DISCUSSION

The phytochemical screening of ethanol extract of *P. chinensis* whole plant revealed the presence of alkaloid, catechin, coumanin, flavonoid, tannin, saponin, steroid, phenol, glycoside, terpenoid and xanthoprotein. Acute toxicity study revealed the non-toxic nature of the ethanol extract of *P. chinensis* whole plant. Table 1 shows the levels of blood glucose, serum insulin, urea, creatinine and glycosylated haemoglobin of normal, diabetic rats and drug treated rats. The alloxan induced rats elicited significant rise in blood glucose from 83.16 to 231.84mg/dl (P<0.01) and a significant decrease in serum insulin from 14.27 to 5.21 (P<0.01). On the contrary, diabetic rats treated with ethanol extract of *P. chinensis* whole plant exhibited decrease in blood glucose and increase in serum insulin significantly (P<0.05) at a dose of 200mg/kg body weight. It was observed that ethanol extract of *P. chinensis* reversed these effects in diabetic rats. Glibenclamide, reference standard produced a significant (P<0.01) reduction in blood glucose and increase insulin compared to diabetic control.

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose; insulin is secreated [28]. Alloxan is one of the usual substances used for the inducing diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas [29, 30]. Alloxan causes a massive reduction in insulin release by the destruction of β-cells of the islets of langerhans, there by inducing hyperglycaemia [31]. Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose. The possible mechanism by which ethanol extract of *P. chinensis* whole plant brings about its hyperglycemic action may be induction of pancreatic insulin secretion from β-cells of islets of langerhans or due to enhanced transport of blood glucose to peripheral tissue [32]. Earlier many plants have been studied for their hypoglycemic and insulin release stimulatory effects [33-36].

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. chinensis* 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. Alloxan is taken as inductions of an abnormal glomerular fraction where a simple injection of cisplation at a dose of 5mg/kg body weight in rabbits caused a marked reduction in the glomerular filtration rates, which was accompanied by an increase in the creatinine level, indicating the induction of acute renal failure. It is confirmed that there is a significant increase in serum creatinine in albino rats 14 days after alloxan administration. The present results show that, the treatment with ethanol extract of *P. chinensis* whole plant was effective in preventing alloxan induced increase in serum creatinine level when compared in the control. Alloxan induced diabetic rats showed significant increase (P<0.01) glycosylated haemoglobin (HBA_1C) level compared with normal rats. The ethanol extract of *P. chinensis* whole plant treated rats showed a significant decrease (P<0.05) in the content of glycosylated haemoglobin. Glycosylated haemoglobin determinations are self monitoring of blood glucose therefore play on important complementary roles for the management of diabetes mellitus [37].

The levels of serum protein, albumin and globulin of control, alloxan induced diabetic rats

Table -1: Effect of ethanol extract of *P.chinensis* whole plant on the serum insulin, glucose, urea, creatinine and HBA_1C 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_1C(%)
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	108.26 ±1.26 [a]	10.36± 0.36 [a]	18.29± 0.32 [a]	0.86 ±0.06 [a]	6.24 ±0.26 [a]
IV	133.84±2.08* [a]	11.96±0.26 [a]	14.54±0.26 [a]	0.81±0.03	4.99±0.24 [a]
V	94.66±1.84 [aa]	13.63±0.18 [aa]	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 *P.chinensis* 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	6.24 ±0.13	3.24 ±0.12	3.00 ±0.08	24.61± 0.78	26.11± 0.32	154.16± 2.21
Group IV	7.94±0.14	4.44±0.17	3.50±0.21	15.11±0.36	22.14±0.33	129.63±1.14
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. chinensis* 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 – (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	110.3±41.06	128.3±61.02	18.26±1.36	66.56±1.36	21.36±1.14	189.32±2.31
IV	96.16±1.13[aa]	73.21±1.13[aa]	21.74±1.23[a]	57.78±1.22[a]	15.64±1.27	153.58±2.35
V	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
VI	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 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.

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). On administration of ethanol extract of *P. chinensis* to the diabetic rats, the levels of protein, albumin and globulin were found to be restored in normal. These results were in accordance with the effects of *Wattakaka volubilis* [38], *Senna auriculata* [39] and *Pterocarpus marsupium* [34] and *Eugenia floccose* [35].

Table 2 summarized the effect of alloxan on the activity of the hepatic marker enzymes in serum. In the present study, 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 [40]. AST and ALT were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats [41]. In this study, the ethanol extract of *P. chinensis* 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 [42].The restorations of SGPT and SGOT to their respective normal levels after treatment with both glibenclamide and ethanol extract of *P. chinensis,* further strengthen the antidiabegenic effect of this extract. Moreover, 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.

In addition to the assessment of SGPT and SGOT levels during diabetes the measurement of enzymatic activities of phosphatases such as acid phosphatase (ACP) and alkaline phosphatase (ALP) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants. In the present study, serum ALP increased in alloxan induced diabetic rats (Table 2). Elevated level of this enzyme in diabetes may be due to extensive damage to liver in the experimental animal by alloxan. Treatment with ethanol extract of *P. chinensis* in alloxan induced diabetic rats produces a decline in ALP level.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. chinensis* (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. chinensis* and glibenclamide to the diabetic rats, HDL-C level was found to be restored to normal. The level of serum lipid profiles are usually raised in diabetic rats in the present study and such elevation represents risk factor for coronary heart diseases [43]. The hypolipidaemic effect may be due to inhibition of fatty acid synthesis [44]. In normal metabolism insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia. The significant reduction of serum lipid levels in diabetic rats after *P. chinensis* treatment may be directly attributed to improvements in insulin levels. Phospholipids are present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar plasma environment and non-polar lipoprotein of lipoprotein core [45]. Increased phospholipids levels in tissues were reported by Venkateswaran *et al* [46]; Pari and Satheesh [47] in streptozotocin diabetic rats. Administration of ethanol extract of *P. chinensis* whole plant and glibenclamide decreased the levels of phospholipids.

4. CONCLUSION

The present study has shown that the ethanol extract of *P. chinensis* whole plant have antidiabetic and antihyperlipidaemic effects. From this study, the ethanol extract of *P. chinensis* whole plant is beneficial in controlling the blood glucose level, improves the lipid metabolism. The possible antidiabetic activity of the extracts might be due to stimulation of residual pancreatic insulin or by increasing peripheral utilization of glucose. Glycosides, flavonoids, tannins and alkaloids are active ingredients of hypoglycemic plant [48]. Flavonoids are reported to regenerate the damaged pancreatic beta cells [49]. Phenols have found to be effective antihyperglycemic agents [50]. In the present study, the phytochemical analysis of ethanol extract of *P. chinensis* clearly pointed out the presence of above said active phytochemicals. Further identification and isolation of these constituents may be fruitful.

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