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ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFECT OF *PARMELIA PERLATA*. ACH. IN *ALLOXAN INDUCED DIABETIC RATS*

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ABSTRACT

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia and alteration in the carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion or insulin action. In the present study antihyperlipidemic activity of *Parmelia perlata*. Ach. has been evaluated in alloxan induced diabetic albino rats. The aqueous extract of the selected plant was administered at dose levels of 200mg and 400mg/kg body weight for 60 days. After the experimental period the blood and tissue samples were collected and subjected to various biochemical and enzymic parameters. There were profound alteration in fasting blood glucose, serum insulin, glycosylated hemoglobin (HbA_1C) and liver glycogen levels in alloxanized rats. Glucose-6-phosphatase, glucokinase, and fructose 1-6 bisphosphatase activity were also altered in diabetic rats. Administration of plant extract significantly ($P<0.05$) reduced the fasting blood glucose and HbA_1C level and increased the level of plasma insulin. The activities of glucose metabolizing enzymes were also resumed to normal. There was a profound improvement in serum lipid profiles by reducing serum triglyceride, cholesterol, LDL, VLDL, free fatty acids, phospholipids and increasing the HDL level in a dose dependent manner. The effects of leaf extract were compared with standard drug glibenclamide (600µg/Kg bw). The results indicate that *Parmelia perlata*. Ach., Linn. could be a good natural source for developing an antidiabetic drug that can effectively maintained the blood glucose levels and lipid profile to near normal values.

Keywords: *Parmelia perlata*. Ach., Insulin, HbA_1C, Glucose-6-phosphatase, Glucokinase, Cholesterol, Triglycerides, HDL, LDL.

INTRODUCTION

Diabetes is a group of chronic metabolic disorder without a cure and it is associated with significant morbidity and mortality. The complications of diabetes mellitus are mainly due to severe hyperglycemia. Chronic complications are characterized by the dysfunction of various organs, especially the eyes (retinopathy), kidneys (nephropathy), nerves (neuropathy), heart (cardiomyopathy) and blood vessels [1]. Diabetes mellitus is considered to be the world's largest growing metabolic disorder and about 30 million people around the world suffer from this disorder. Treatment of diabetes with synthetic drugs are generally not preferred because of prohibitive cost of treatments, side effects on prolonged usage and development of resistance. Hence the development of traditional or alternative medicine has become indispensible.

About 60% of the world population use Traditional medicines derived from medicinal plants. In the past few years, there has been an exponential growth in the field of herbal medicine. Herbal drugs are gaining momentum both in developing and developed countries because of their natural origin and less side effects [2]. Herbal drugs constitute an important part of the traditional medicine and literature shows that there are more than 1200 plant species showing antidiabetic activity. The plant species mentioned in the ancient texts of Ayurveda and other Indian systems of medicines may be explored with the modern scientific approaches in order to develop a better leads in the health care [3]. *Parmelia perlata* Ach. is small lichen belonging to family *Parmeliaceae*, is commonly found t**hroughout India, in rocky areas, old tree trunks.** Seen especially in Himachal Pradesh, Kerala and Bengal in India. It is known as Shila pushpha, Saileya in Sanskrit, **Chadeela, Pathar ka phool** in Hindi and Stone flower in English, Kalpassi in Tamil. The lichen is astringent, cooling, anti-inflammatory and aphrodisiac. It is useful in curing sores, boils, inflammations head ache and skin problems. It is kappa and pitta suppressant. It relieves pain and also promotes early healing of wounds. 'Saileya' (*Parmelia perlata* Ach) form ingredients of 'Diabecon' a herbomineral preparation prescribed in diabetes mellitus [4] *P.perlata* Ach.. has also been used in the management of obesity to maintain the lipid profiles. As altered lipid profile occurs in diabetes, any drug source with hypolipedimic activity can also be screened for the hypoglycemic potential. So these herbal sources were selected and evaluated for their antidiabetic antihyperlipidemic potentials using experimental models.

MATERIAL AND METHODS

Preparation of plant extract

Parmelia perlata Ach. were obtained from places in and around Trichy identified and authenticated with the herbarium specimen of RAPINAT herbarium of St. Joseph's College, Trichy, Tamilnadu, India. The collected drug materials were coarsely powdered with electrical blender

200 g of *Parmelia perlata* Ach. coarse powder was taken and extracted with water. To one part of the material six parts of water was added, boiled and reduced to one third and the filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to pre-clinical screening. The percentage yield of extract was 46.

Experimental Animals

Healthy adult wistar strain of albino rats of either sex, weighing 150-200 g was used as experimental models. Animals were kept in well-ventilated cages and fed with standard rat chow pellet obtained form Sai Durga Food and Feeds, Bangalore, India and water *ad libitum*. Animals were maintained under standard laboratory conditions (Temperature 24-28ºC, relative humidity 60-70%). After obtaining necessary clearance from the committee (approval No: 790/03/ac/CPCSEA), the studies were conducted according to the ethical guidelines of CPCSEA.

Alloxan induction

Diabetes mellitus was induced in normoglycemic albino rats kept under starvation for 16 hours to group II, III, IV and V. 150mg/kg body weight of alloxan monohydrate was dissolved in physiological saline and injected intraperitoneally (IP). This dose of alloxan produced persistent hyperglycemia after 4 days as revealed by determination of sugar levels by the analysis of blood and urine samples [5].

Experimental design

The rats were divided into five groups each comprising of six rats.

Group I- Normal control
Group II- Animals treated with alloxan monohydrate in normal saline at a dosage of 150mg/kg body weight IP.
Group III - Animals were treated as in Group II. After 4 days of alloxan induction, treated with *Parmelia perlata* Ach. aqueous extract **(PPAE)**- 200mg/kgbody weight, orally for 60days.
Group IV - Animals were treated as in Group II. After 4 days of alloxan induction, treated with *Parmelia perlata Ach*. aqueous extract **(PPAE)**- 400mg/kg body weight orally for 60 days.
Group V - Animals were treated as in Group II. After 4 days of alloxan induction, treated with standard drug glibenclamide - 600µg/kg body weight orally for 60 days.

Collection of blood and liver from the rat

At the end of the experimental period, animals were sacrificed by cervical decapitation. Blood was collected and serum was separated by centrifugation (for 15min at 2000rpm). The liver were dissected out and washed in ice-cold saline. Tissues were cut into small pieces and homogenized, in 0.1M phosphate buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for various biochemical and enzymatic analysis.

The parameters studied

Fasting blood glucose by folin-Wu's method [6], glycogen [7], plasma Insulin [8], Glycosylated hemoglobin (HbA1C) [9] and protein were analyzed in blood sample. Glucokinase [10], Glucose-6-phosphatase [11&12], fructose 1-6 bisphosphatase [13] in liver tissues. Serum lipid profiles evaluation includes cholesterol [14], triglyceride [15], phospholipids [16&12], free fatty acids [17], HDL [18], LDL [18], and VLDL [18].

Statistical analysis

All the results were expressed as mean ± S.E.M. The data were statistically analyzed by one – way analysis of variance (ANOVA) between plant extract treated groups and disease control group. P values <0.05 were considered as significant.

RESULTS

The effect of PPAE on blood glucose, glycosylated haemoglobin (HbA_1C) serum insulin and liver glycogen were shown in **Table: 1**. Administration of alloxan (150 mg/kg; b.w; I.P) showed an elevated level of fasting blood glucose and glycosylated haemoglobin (HbA_1C) and reduction in serum insulin and liver glycogen compared to normal rats, which might be due to the destruction of beta-cells. Oral administration of aqueous extract of *Parmelia perlata* Ach (200 mg/kg; b.w and 400 mg/kg; b.w) for 60 days showed a significant reduction ($P < 0.05$) in blood glucose and HbA_1C levels, when compared with diabetic control animals. Serum insulin and glycogen levels were also restored to near normal.

Table 1: Levels of blood glucose, glycosylated haemoglobin (HbA_1C) serum insulin and liver glycogen in control and PPAE treated rats for 60 days

Groups	Parameters	Blood Glucose (mg/dl)	Serum insulin (µU/ml)	HbA_1C (%)	Glycogen (mg/g tissue)
I	Control	86.4 ± 9.20	6.4 ± 2.61	2.6 ± 0.21	46.5 ± 8.91
II	Diabetic	270.4 ± 21.24*	4.3 ± 0.93*	6.6 ± 1.41*	11.7 ± 0.91 *
III	Diabetic + PP treated (200mg/Kg)	211.7 ± 18.26**	4.9 ± 0 .74**	5.9 ±1.62**	22.7 ± 5.05 **
IV	Diabetic + PP treated (400mg/Kg)	101.2 ± 12.73**	6.1 ±0.97 **	2.9 ± 0.55 **	37.5 ± 7.25 **
V	Diabetic + Glibenclamide (600µg/Kg)	83.4 ± 7.85	5.7 ± 0.64	3.1 ± 0.76	41.3 ± 8.05

Values are Mean ± SEM (n=6)

Inter-group comparison

*P < 0.05.statistically significant when Group II and Group I were compared

**P < 0.05.statistically significant when Group III, & IV were compared with Group II,

The activity of glycolytic and gluconeogenic enzymes in control and PPAE treated rats were shown in **Table: 2.** Alloxanized group II rats showed marked alteration in the activity of glucokinase, Fructose – 1 – 6 bisphosphatase and glucose – 6 – phosphatase. Treatment with PPAE at a dose level of 200mg/Kg bw and 500mg/Kg bw increases the activity of glucokinase activity and reduces the activity of Fructose – 1 – 6 bisphosphatase and glucose – 6 – phosphatase. The effect was found to be higher in higher dose (400mg/Kg) than the lower dose (200mg/Kg).

Table 2: Effect of PPAE on the activities of glycolytic and gluconeogenic enzymes in control and treated rats

Groups	Parameters	Glucokinase (µ moles of Glu- 6- PO_4 formed/ min/ mgprotein)	Fructose – 1- 6 - bisphosphatase (µ moles of PO_4 liberated/ min/ mg protein)	Glucose –6 – phosphatase (nmoles of Pi liberated/ min/ mg protein)
I	Control	136.7 ± 20.26	26.4 ± 5.61	7.7± 1.21
II	Diabetic	96.2 ± 12.05*	124.7 ± 11.23*	15.4 ± 2.24*
III	Diabetic + **PPAE** treated (200mg/Kg)	102.7 ± 17.24 **	99.7 ± 8.52 **	12.7 ± 2.11**
IV	Diabetic + **PPAE** treated (400mg/Kg)	128.5 ± 16.05 **	33.7± 8.11 **	9.7 ± 1.66 **
V	Diabetic + Glibenclamide (600µg/Kg)	134.6 ± 18.65	32.4± 6.62	7.1 ± 1.67

Values are Mean ± SEM (n=6)

Inter-group comparison

*P < 0.05.statistically significant when Group II and Group I were compared.

**P < 0.05.statistically significant when Group III, & IV were compared with Group II,

The effect of PPAE on serum total cholesterol, triglyceride (TGL), phospholipids (PL) and free fatty acid (FFA) were summarized in **Table 3.** Animals treated with test drug showed a profound (P<0.05) decrease in the levels of serum cholesterol, TGL, PL and FFA.

Table 4 Shows the lipoprotein profiles of normal, disease control and plant treated group of animals. The levels of VLDL and LDL were elevated markedly in group II diabetic animals. On treatment with plant drug, the above parameters brought back to near normal. HDL that was considered as good cholesterol was decreased in untreated group II of animals and on treatment with *Parmelia perlata* Ach aqueous extract it was found that HDL – good cholesterol was elevated significantly (P<0.05) and it was dose dependent

Table 3: Serum lipid profiles of untreated and PPAE treated rats for 60 days

Groups	Parameters	Cholesterol (mg/dl)	TGL (mg/dl)	PL (mg/dl)	FFA (mg/dl)
I	Normal control	60.8 ± 7.42	68.4 ± 6.81	58.2 ± 5.14	44.4± 7.52
II	Diabetic control	141.5 ± 23.94 *	168.6 ± 22.56 *	122.3 ± 14.81 *	110.7± 19.32 *
III	Diabetic + PPAE treated (200mg/Kg bw)	122.4 ± 17.25**	121.7 ± 19.02 **	111.6 ± 16.42 **	96.5± 9.28 **
IV	Diabetic+ PPAE treated (400mg/Kg bw)	79.5± 9.44 **	72.1 ± 6.53 **	61.2± 5.05 **	48.7± 6.71 **
V	Diabetic + Glibenclamide treated (600µg/Kg bw)	59.8 ± 9.20	64.7 ± 7.22	62.3± 9.57	47.6± 8.62

Values are Mean ± SEM (n=6)

Inter-group comparison

*P < 0.05.statistically significant when Group II and Group I were compared.

**P < 0.05.statistically significant when Group III, & IV were compared with Group II.

Table 4: Serum lipoprotein profile of untreated and treated rats after oral administration of PPAE for 60 days

Groups	Parameters	HDL -Cholesterol (mg/dl)	LDL -Cholesterol (mg/dl)	VLDL -Cholesterol (mg/dl)
I	Normal control	61.5 ± 6.42	41.5 ± 5.01	13.9 ± 1.14
II	Diabetic control	22.5 ± 6.04 *	69.3 ± 9.52 *	30.3 ± 3.01 *
III	Diabetic + PPAE treated (200mg/Kg bw)	29.6 ± 5.22**	55.3 ± 7.26 **	24.2± 3.25 **
IV	Diabetic + PPAE treated (400mg/Kg bw)	50.7 ± 7.41 **	43.1 ± 5.51 **	14.4± 2.05 **
V	Diabetic + Glibenclamide treated (600µg/Kg bw)	52.5 ± 6.38	44.7 ± 6.54	17.2 ± 4.37

Values are Mean ± SEM (n=6)

Inter-group comparison

*P < 0.05.statistically significant when Group II and Group I were compared.

**P < 0.05.statistically significant when Group III, & IV were compared with Group II.

DISCUSSION

Diabetes mellitus is a chronic metabolic disorder that has risen dramatically over the past 2 decades. Although the prevalence of both type I and II diabetes mellitus are increasing world wide, the prevalence of type II diabetes is expected to rise rapidly. In spite of this, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs have paved way to increase the emphasis on the use of plant based medicines for a wide variety of human ailments and medications. In this present investigation, effect of aqueous extract of *Parmelia perlata* Ach. has been evaluated for its antidiabetic and antihyperlipidemic potential. Alloxan is a potent diabetogen that is reduced to dialuric acid which is then auto oxidized back to alloxan resulting in the production of H_2O_2, O_2, O^{2-} and hydroxyl radicals and causes damages to the beta-cells of islets of langerhans [19]. This causes a profound decrease in insulin level and consequent increase in fasting blood glucose level in disease control animals (Group II) **(Table: 1).** Administration of test drug for 60 days (Group III& IV) was found to regenerate the pancreatic beta-cells which results in the normal secretion of insulin. Insulin, the potent hypoglycemic hormone thereby reduces the blood glucose level significantly (P < 0.05).

Glycosylated hemogolobin (HbA_1C) is formed progressively and irreversibly when the blood glucose level is elevated. HbA_1C increases over a period of time and does not dissociate easily, it reflects the real blood glucose level in diabetes hence HbA_1C is used as an exceptional marker of overall glycemic control [20]. Group II animals showed elevated levels of HbA1C (P < 0.05). Due to insulin deficiency in diabetic rats the cells utilizes glycogen rather than glucose for their energy [21]. Hence the diabetic rats showed decreased level of liver glycogen compared to the normal rats **(Table: 1).** Plant treated groups (III and IV) showed a marked increase (P < 0.05) in glycogen level and significant decrease in HbA_1C levels. The depletion of glycogen in the liver tissue might be prevented by the stimulation of insulin release from β - cells that in turn activates the glycogen synthase system and thereby helps in the resumption of liver glycogen level. Reference drug also increased the glycogen level but not as effective as plant extract treatment.

Glucokinase is the key enzyme catalysing glucose phosphorylation in liver. Impairment of glucokinase activity suggests the impaired oxidation of glucose via glycolysis causes its accumulation resulting in hyperglycemia. Insulin increases hepatic glycolysis by increasing the activity and amount of the key enzyme – glucokinase [22]. The diabetic rats showed decreased activity of glucokinase compared to normal rats. Groups (III, and IV) treated with the *Parmelia perlata* Ach. extract (200 mg/kg; b.w and 400 mg/kg; b.w) showed a significant increase (P < 0.05) in the activity of glucokinase. Glucose-6-phosphatase plays a vital role in the blood glucose homeostatis. When the blood glucose level falls, liver is releases glucose into the circulation rapidly, where it serves as a fuel for other tissues that

lack the ability to make glucose [23]. Increased hepatic glucose output is a major cause of the fasting hyperglycemia that characterizes diabetes. Group III and IV animals that received 200 mg/kg; b.w and 400 mg/kg; b.w for 60 days showed a remarkable decrease in the activity of Glucose-6-phosphatase and Fructose – 1-6-bisphosphatase **(Table: 2).** This suggest that the aqueous extract of *Parmelia perlata* Ach. is a potent antidiabetic agent.

The alloxan induced diabetic rats showed a remarkable increase ($P < 0.05$) in the levels of lipid profiles such as total cholesterol (TC), Triglycerides (TGL) and lipoproteins such as LDL- cholesterol, VLDL-cholesterol **(Table: 3 & 4)**. There was a marked reduction in HDL-cholesterol compared to normal control. Studies in human and animals demonstrated that the alteration of lipid profiles in conditions of diabetes represents a risk factor for cardiovascular diseases. Elevated levels of TC and LDL-cholesterol levels in diabetes are the cause of coronary heart disease. The high concentration of serum lipids in diabetes mellitus is essentially due to an increase in the mobilization of free fatty acids from the peripheral fat depots. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [24].

Breakdown of fatty acids for energy results in increased production of acetyl CoA which may take up the pathway of cholesterol biosynthesis. TGL is the source of energy during starvation. In diabetes mellitus as glucose is not taken up by the cells. The cells are under starvation and depend on TGL for energy. Hence there is a rapid mobilization of TGL from tissues in diabetic rats (Group II) **(Table: 3 & 4)**.). It was observed that treatment with *Parmelia perlata* Ach. showed a significant reduction ($P < 0.05$) in the tissue lipid profiles (TC and TGL) and lipoprotein (LDL and VLDL). The level of HDL-cholesterol was increased significantly in groups III and IV thus helped in retarding the secondary complications of diabetes mellitus.

CONCLUSION

Many indigenous Indian medicinal plants are used as remedies against various diseases. In the present scenario herbal medicines and herbal research is coming to lime light. The present investigation evaluates the antidiabetic and antihyperlipidemic efficacy of *Parmelia perlata* Ach. Further investigations to identify the active principles are obviously needed together with a detailed evaluation on the mechanism of action of *Parmelia perlata* Ach in combating altered glycemic and lipid profile in diabetic condition.

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