

ANTIDIABETIC AND ANTIHYPERLIPIDAEMIC ACTIVITY OF *SONERILA TINNEVELLIENSIS* FISCHER WHOLE PLANT IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Objective: The ethanol extract of *Sonerila tinnevelienseis* Fischer whole plant was investigated for its antidiabetic and antihyper lipidaemic effect in Wistar Albino rats.

Methods: Diabetes was induced in Albino rats by administration of alloxan monohydrate (150 mg/kg, i. p). The ethanol extracts of *S. tinnevelienseis* at a dose of 200 and 400 mg/kg of body weight were administered at single dose per day to diabetes induced rats for a period of 14 d. The effect of ethanol extract of *S. tinnevelienseis* whole plant extract on blood glucose, plasma insulin, creatinine, urea, 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.

Results: The ethanol extract of *S. tinnevelienseis* 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 plasma insulin ($p < 0.05$) in the diabetic rats.

Conclusion: The ethanol extracts of *S. tinnevelienseis* whole plant possesses significant antidiabetic and antihyperlipidaemic effects in alloxan induced diabetic rats.

Keywords: *Sonerila tinnevelienseis*, Diabetes mellitus, Alloxan, Hyperglycemia.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders with one common manifestation-hyperglycemia [1]. Chronic hyperglycemia causes damage to eyes, kidneys, nerves, heart and blood vessels [2]. It is caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It results either from inadequate secretion of hormone insulin, an inadequate response of target cells to insulin, or a combination of these factors. This disease requires medical diagnosis, treatment and changes in life style. It is projected to become one of the world's main disablers and killers within the next 25 y. The management of diabetes is a global problem until now and successful treatment is not yet discovered. There are many synthetic medicines developed for patients, but it is the fact that it has never been reported that someone had recovered totally from diabetes [3]. The modern oral hypoglycaemic agents produce undesirable and side effects. Thus, alternative therapy is required; a need of an hour is to shift towards the different indigenous plant and herbal formulations [4].

More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances. Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes. One of the great advantages of medicinal plants is that these are readily available and have very low side effects. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information reports about 800 plants that may possess antidiabetic potential [5]. Several herbs have shown antidiabetic activity when assessed using presently available experimental techniques [6].

Sonerila, with between 100 and 175 species [7], is the largest genus in the Sonerileae. It is the only consistently trimerous genus in the family and as such easily diagnosed. Leaf extract of *Sonerila tinnevelienseis* was orally administered to cure body swelling by

kanikaran [8]. A handful of leaves consumed on an empty stomach once in a day for 12 to 15 d to get relief from rheumatic complaint [9]. Taking into consideration of the medicinal importance of *Sonerila tinnevelienseis*, the ethanol extract of the whole plant of *Sonerila tinnevelienseis* were analyzed for their antidiabetic and anti hyperlipidaemic activity in alloxan induced diabetic rats.

MATERIALS AND METHODS

Collection of plant material

Whole plant of *Sonerila tinnevelienseis* was collected from Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu with the help of local flora, the specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for anticancer activity

The whole plants of *S. tinnevelienseis* were cut into small pieces, washed and dried at room temperature; the dried whole plant was powdered in a Wiley mill. Hundred grams of powdered whole plant was separately packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract of the whole plant was used for antidiabetic activity.

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 [10] (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 5 mg/kg body weight by gastric intubations and observed for 14 d. 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 experimental diabetes

Rats were induced diabetes by the administration of simple intra peritoneal dose of alloxan monohydrate (150 mg/kg) [11]. 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 *S. tinneveli* whole plant (200 mg/kg body weight)

Group IV: Diabetic rats given ethanol extract of *S. tinneveli* whole plant (400 mg/kg body weight)

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

Biochemical analysis

The animals were sacrificed at the end of experimental period of 14 d by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 min. Serum glucose was measured by the O-toluidine method [12]. Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit [13] Urea estimation was

carried out by the method of Varley [14]; serum creatinine was estimated by the method of Owen *et al.* [15]. Glycosylated haemoglobin (HbA_{1c}) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan [16]. Serum total cholesterol (TC) [17], total triglycerides (TG) [18], low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) [19], high density lipoprotein cholesterol (HDL-C) [20] and phospholipids [21] were analyzed. Serum protein [22] 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 [23]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong [24] in the normal, diabetic induced and drug treated rats.

Statistical analysis

The data was analyzed using student's t-test statistical methods. For the statistical tests a *p* values of less than 0.001, 0.01 and 0.05 was taken as significant.

RESULTS

In acute toxicity studies animals treated with ethanol extract of the whole plant of *S. tinneveli* did not show any toxic symptoms or mortality when doses upto 2000 mg/kg body weight by oral route. This indicated that the extract was found to be safe at the tested dose level. Hence 200 mg/kg and 400 mg/kg of the doses were selected for the *in vivo* studies. The present investigation indicates that ethanol extracts of the whole plant of *S. tinneveli* showed significant antidiabetic activity in rats.

In the present study, alloxan induced diabetic rats showed significant (*p*<0.01) reduction in body weight. Administration of ethanol extracts of the whole plant of *S. tinneveli* (200 and 400 mg/kg) and glibenclamide (600 mg/kg) significantly (*p*<0.05) increased the body weight within 14 d (table 1). Fasting blood glucose levels of the diabetic control rats were higher than those of normal rats. A significant (*p*<0.05) dose dependent decrease in blood glucose levels was observed in the diabetic treated group from an initial level of 208.46 mg/dL to the level of 113.15 mg/dL and from 216.90 mg/dL to 109.34 mg/dL after the treatment at a dose of 200 mg/kg and 400 mg/kg respectively for 14 d (table 1).

Table 1: Effect of *S. tinneveli* whole plant extracts on the body weight and fasting blood glucose in normal, diabetic and diabetic treated rats

Group	Mean initial body weight (g)	Mean final body weight (g)	Mean weight Gain (G↑)/loss(L↓) (g)	Fasting Blood glucose (mg/dl)		
				0-Day	7-days after	14-days after
I	198.50±5.19	206.16±4.95	7.66	81.65±2.45	78.18±1.94	83.65±1.68
II	204.16±6.13	191.65±3.84ns	12.51	231.66±6.84***	229.16±4.65***	234.26±6.83***
III	189.65±3.15	194.31±2.96ns	4.66	208.46±5.13***	124.81±3.14ns ^{aa}	113.15±3.91 ^{aa}
IV	194.50±2.15	198.56±3.95	4.06	216.90±6.54	119.34±5.86 ^{aaa}	109.34±6.21 ^{aaa}
V	206.40±5.81	210.16±5.28	3.76	207.18±5.84***	109.65±5.13 ^{aa}	103.56±5.91 ^{aa}

Each Value is SEM of 6 animals: Comparison made between normal control to diabetic control and drug treated groups ***p*<0.001; Comparison made between diabetic control to drug treated groups level of significance ^{aa}*p*<0.01; ^{aaa}*p*<0.001; ns-not significant.

Table 2: Effect of *S. tinneveli* whole plant extracts on the serum insulin, glucose, urea, creatinine and HbA_{1c} level of normal, diabetic, diabetic treated rats

Group	Insulin (MIU/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Glycolyted Hb
I	16.68±0.81	83.65±1.68	11.96±0.91	0.93±0.05	3.94±0.17
II	6.34±0.13***	234.26±6.83***	34.91±2.68*	3.42±0.06*	9.34±0.26**
III	14.28±0.95 ^{aa}	113.15±3.91 ^{aa}	19.28±1.54 ^{ns}	1.98±0.07 ^{ns}	5.13±0.11 ^a
IV	16.35±1.08 ^{aaa}	109.34±6.21 ^{aa}	14.36±0.93 ^a	0.74±0.03 ^a	4.68±0.65 ^{aa}
V	17.24±0.91 ^{aaa}	103.56±5.91 ^{aa}	13.14±1.21 ^{aa}	0.84±0.07	5.04±0.21 ^{aa}

Each Value is SEM of 6 animals: Comparison made between normal control to diabetic control and drug treated groups **p*<0.05; ***p*<0.01; ****p*<0.001, Comparison made between diabetic control to drug treated groups level of significance ^a*p*<0.05; ^{aa}*p*<0.01; ^{aaa}*p*<0.001; ns-not significant

Table 2 shows the levels of blood glucose, serum insulin, urea, creatinine and glycosylated haemoglobin of normal, diabetic control and drug treated rats. There was a significant ($p<0.01$) increase blood glucose level in alloxan induced diabetic rats (Group II) when compared with normal rats (Group I). Administration of whole plant of *S. tinneveli* (Group III and IV) and glibenclamide (Group V) tended to bring the parameters significantly ($p<0.05$; $p<0.01$) towards the normal. Serum insulin level of diabetic control group was significantly ($p<0.01$) decreased when compared to the normal control group (Group I). The extract and glibenclamide group of diabetic rats significantly ($p<0.01$) increased the serum insulin. A significant ($p<0.01$) elevation in urea and creatinine was observed in alloxan induced diabetic rats (Group II) when compared to control rats. The *S. tinneveli* whole plant was administered orally to diabetic rats for 14 days reversed the urea and creatinine level to near normal. Administration of ethanol

extracts of *S. tinneveli* whole plant (400 mg/kg) and glibenclamide significantly ($p<0.05$) reduced HbA1C level compared to diabetic control rats.

The level of total protein, albumin, globulin and liver marker enzymes such as SGPT, SGOT and ALP in the serum of diabetic rats are presented in Table-3. Significant reductions in serum protein, albumin and globulin were observed in alloxan induced diabetic rats (Group II) when compared to control rats (Group I). On administration of ethanol extract of the whole plant of *S. tinneveli* to the diabetic rats, protein, albumin and globulin levels were found to be restored in normal. Also the SGPT, SGOT and ALP levels were elevated significantly in alloxan induced diabetic rats compared to control rats. Both the doses of whole plant of *S. tinneveli* extracts and glibenclamide treatment significantly reduced above parameters compared to diabetic control rats.

Table 3: Effect of *S. tinneveli* whole plant extracts on the serum protein, albumin, globulin, SGOT, SGPT and ALP level of normal, diabetic induced, and drug treated rats

Groups	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
I	8.31±0.16	4.89±0.21	3.42±0.32	19.33±0.91	20.84±0.86	178.31±4.27
II	6.88±0.24*	3.94±0.12 ^{ns}	2.94±0.52	46.13±1.26*	48.04±3.62*	216.62±3.16**
III	7.91±0.16 ^{ns}	4.53±0.26	3.38±0.61	22.65±1.18 ^a	19.54±2.81 ^a	186.27±2.91 ^{ns}
IV	8.04±0.13	4.61±0.16	3.43±0.46	18.27±1.36 ^{aa}	21.63±1.93 ^{aa}	194.11±3.65
V	8.14±0.18 ^a	4.91±0.22	3.23±0.13	18.36±0.93 ^a	21.68±1.38 ^a	166.37±2.13 ^a

Each value is \pm SEM of 6 animals: Comparison made between normal control to diabetic control and drug treated groups * $p<0.05$; ** $p<0.01$, Comparison made between diabetic control to drug treated groups level of significance ^a $p<0.05$; ^{aa} $p<0.01$

Table 4 shows the levels of TC, TG, LDL-C, VLDL-C, HDL-C and phospholipid in the serum of diabetic rats showed significantly ($p<0.01$) increased serum lipid profiles except HDL-C when compared with normal rats. The ethanol extracts of *S. tinneveli* whole plant treated rats showed a significant ($p<0.01$, $p<0.05$) 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 with normal rats. Administration of ethanol extracts of *S. tinneveli* whole plant and glibenclamide to the diabetic rats. HDL-C level was found to be restored to normal.

Table 4: Effect of *S. tinneveli* whole plant extracts on the serum lipid profile of normal, diabetic induced, and drug treated rats

Groups	TC (mg/dl)	TG(mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	PL (mg/dl)
I	124.61±2.54	138.69±3.16	64.26±2.87	27.73±1.34	32.62±1.17	178.90±2.45
II	182.92±2.45**	194.67±2.24**	124.36±3.65**	38.93±1.75*	19.63±0.94*	230.79±2.68**
III	142.65±1.84 ^{ns}	158.24±1.83 ^a	83.69±2.93 ^{aa}	31.65±1.08 ^{ns}	27.31±1.64 ^{ns}	194.96±2.33 ^a
IV	128.19±1.93 ^{aa}	139.16±1.08 ^a	62.09±3.08 ^{aa}	27.83±2.19 ^a	38.26±1.32 ^a	182.08±4.29 ^a
V	120.89±1.24 ^{aa}	129.38±1.78 ^{aa}	58.82±2.96 ^{aa}	25.88±1.56 ^a	36.19±1.18 ^a	175.59±2.16 ^a

Each Value is SEM of 6 animals: Comparison made between normal control to diabetic control and drug treated groups * $p<0.05$; ** $p<0.01$, Comparison made between diabetic control to drug treated groups level of significance ^a $p<0.05$; ^{aa} $p<0.01$

DISCUSSION

Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Insulin is a key player in the control of glucose homeostasis. Lack of insulin affects carbohydrate, fat and protein metabolism [25]. Management of diabetes without side effects is still challenge to the medical community.

The present investigation highlights the antidiabetic efficacy of ethanol extract of *S. tinneveli* whole plant. In alloxan induced diabetic rats treated with the plant extract, dose dependent reduction in blood glucose level was observed. Alloxan, a urea derivation and beta cytotocin causes massive destruction of β -cells of the islets of langerhans resulting in reduced synthesis and release of insulin. This leads to hyperglycemia and diabetes [26]. It is well established that sulphonylureas produce hypoglycemia by increasing the secretion of insulin from pancreas and these compounds are active in mild alloxan diabetes (nearly all β -cells have been destroyed) [27]. In diabetic condition, elevated blood glucose, reduction in body weight, polyuria, polydipsia and polyphagia are commonly observed. In the present study, induction of diabetes by alloxan produced rats; observed reduction in body weight was possible due to catabolism of fats and protein [28]. The administrations of ethanol extract *S. tinneveli* whole plant improves body weight compared to diabetic control rats which

indicates preventive effect of *S. tinneveli* whole plant on degradation of structural proteins. The increase in blood glucose level after alloxan administration may be due to insulin deficiency or resistance state in diabetic rats. Administration of ethanol extract of *S. tinneveli* whole plant significantly reduced blood glucose level in diabetic rats which represents reversal of insulin resistance or increasing insulin secretion possibly by regeneration of damaged pancreatic β -cells in alloxan induced diabetic rats [29]. Earlier, many plants have been studied for their hypoglycemic and insulin release stimulatory effects. [30, 31, 32, 33, 34]

In diabetes, elevated levels of serum urea and creatinine are observed which may be due to renal damage caused by abnormal glucose regulation or elevated glucose and glucosylated protein tissue levels [35]. In present study, significant increase in serum urea and creatinine levels were observed in diabetic rats compared to normal control rats which indicate impaired renal function in diabetic rats. The treatment with ethanol extract of *S. tinneveli* whole plant lowered the above parameters significantly compared to diabetic control rats and it showed protective effect of ethanol extract of *S. tinneveli* whole plant on the kidney.

In diabetes, HbA_{1c} is considered as a diagnostic marker and helps to know about degree of protein glycation, long term blood sugar level and correlation of diabetes associated complications [36, 37]. HbA_{1c}

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 [38]. The rate of glycation is proportional to the concentration of blood glucose. In present study, alloxan induced diabetic rats showed significant increase ($p < 0.01$) HbA1c level compared with normal rats. The ethanol extracts of *S. tinneveli* whole plant treated rats showed a significant decrease ($p < 0.05$) in the content of glycosylated haemoglobin that could be due to an important in glycaemic status.

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 enzyme from the tissues and migrating into the circulation by the adverse effect of alloxan [39]. AST and ALT were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats [40]. In this study, the ethanol extract of whole plant of *S. tinneveli* 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 [41]. The restorations of SGPT and SGOT to their respective normal levels after treatment with both glibenclamide and ethanol extract of *S. tinneveli* whole plant, further strengthen the antidiabetic 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. 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 *S. tinneveli* whole plant in alloxan induced diabetic rats produces a decline in ALP level.

On administration of ethanol extract of *S. tinneveli* whole plant 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 [42]. 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 [43]. Thus the results indicate that *S. tinneveli* whole plant 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 make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with polar plasma environment and non-polar lipoprotein core [44]. Increased phospholipids levels in tissues were reported by Venkateswaran *et al.* [45] and Pari and Satheesh [46] in alloxan diabetic rats. Administration of ethanol extract of *S. tinneveli* whole plant and glibenclamide decreased the levels of phospholipids.

CONCLUSION

Medicinal plants have been reported to possess antihyperglycaemic activity. *S. tinneveli* 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. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secretagogues. 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 hypocholesterolemic effect and thus may aid lessening metabolic burden that would have been placed in the liver. In the present study, the phytochemical analysis of ethanol extract of *S. tinneveli* whole plant clearly pointed out the presence of

above said active phytochemicals. It denotes that the antidiabetic effect of ethanol extract of *S. tinneveli* whole plant may be due to the presence of more than one antihyperglycaemic principle and their synergistic effects.

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CONFLICT OF INTERESTS

Declared None

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