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Research Article

HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF ETHANOL EXTRACT OF *SARCOSTEMMA SECAMONE* (L.) BENNET (ASCLEPIADACEAE) IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Objective: To evaluate the hypoglycemic and hypolipidemic effects of ethanol extract of *Sarcostemma secamone* whole plant in alloxan induced diabetic rats.

Methods: Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg i.p). The ethanol extracts of *Sarcostemma secamone* whole plant at a dose of 150 and 300 mg/kg of body weight were administrated at a single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extract of *Sarcostemma secamone* whole plant extract on blood glucose, plasma 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.

Results: In the acute toxicity study, ethanol extract of *Sarcostemma secamone* whole plant was non-toxic at 2000 mg/kg in rats. The increased body weight, decreased blood glucose, glycosylated haemoglobin and other biochemical parameters level was observed in diabetic rats treated with both doses of ethanol extract of *Sarcostemma secamone* whole plant compared to diabetic control rats. In diabetic rats, ethanol extract of *Sarcostemma secamone* whole plant administration, altered lipid profiles were reversed to near normal than diabetic control rats. Conclusion: Ethanol extract of *Sarcostemma secamone* whole plant possess significant hypoglycemic and hypolipidemic activity in diabetic rats.

Keywords: *Sarcostemma secamone*, Hypoglycemic, Hypolipidemic, Alloxan, Glibenclamide, SGOT, SGPT and HbA1C

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder has now become an epidemic, with a World wide incidence of 5% in the general population. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS[1]. According to WHO, the prevalence of diabetes is likely to increase by 35% by the year 2025. Statistical projection about India suggests that the number of diabetes will rise from 15 million in 1995 to 79.4 million by 2025, making it the country with highest number of diabetes in the world[2,3]. Diabetes is a serious metabolic disorder with micro and macrovascular complication that results in significant morbidity and mortality[4].

In conventional therapy, Type 1 diabetes is treated with exogenous insulin and Type 2 with oral hypoglycemic agents (Sulphonylureas, biguanides and thiozolidinediones). But they also have undesired effects associated with their uses[5]. Alternative medicines particularly herbal medicines are available for the treatment of diabetes. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability[6]. The medicinal plants might provide a useful source of new oral hypoglycemic compounds for development of pharmaceutical entities[7]. Few of the plants used for the treatment of diabetes have received scientific or medical scrutiny and even the WHO expert committee on diabetes recommended that this area warrant further attention[8]. Despite the presence of known antidiabetic medicines in the pharmaceutical market, screening for new antidiabetic sources from natural plant is still attractive because they contain substances that have an alternative and safe effect on diabetes mellitus.

Sarcostemma secamone (L) Bennet, is an important medicinal plant belonging to the family Asclepiadaceae. It is used in the traditional systems of medicine for various ailments. The decoction of the plant is useful to gargle for throat and mouth infection. The latex is bitter and used as a vulnerary. Fresh roots are prescribed for jaundice[9-12]. The milky sap forms a wash for ulcers. In combination with turpentine, it is prescribed for itch[13]. The plant is hot, bitter, tonic, expectorant, pungent, dry and indigestible causes flatulence, diuretic, laxative, aphrodisiac, anthelmintic, useful in leucoderma and bronchitis. The juice is used in gleet, gonorrhea, pain in the muscles, cough and given to children as an astringent[14]. Leaf powder stimulates arculatory system, increases secretion of urine and activates uterus[15]. The fruit juice is used in gonorrhea and pain in muscles[14]. The leaves, roots and latex of *Sarcostemma secamone* are employed in treating many diseases like mouth ulcer, sour throat, jaundice and ulcers[16-18]. Realizing the importance and common use of the roots of *Sarcostemma secamone* in the treatment of liver disorder by several tribes in India.

There were no reports on the ability of *Sarcostemma secamone* whole plant on antihyperglycemic and antihyperlipidemic activities. Hence, this study was taken up to investigate the antihyperglycemic and antihyperlipidemic activities of the whole plant of *Sarcostemma secamone* in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant Material

The whole plant of *Sarcostemma secamone* (L.) Bennet was collected from Natural forests of Western Ghats at Thanniparai, Srivilliputhur, Virudhunagar District, Tamil Nadu and identified by the Botanical Survey of India, Coimbatore. A voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

Preparation of plant extract for phytochemical screening and antidiabetic studies

The whole plant of *Sarcostemma secamone* was shade dried at room temperature and the dried whole plant was powdered in a Wiley mill. Hundred grams of powdered 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[19,20]. 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±2ºC) 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*. Study was carried out as per IAFC approval No: 82/PHARMA/SCRI, 2010.

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[21]. 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)[22]. 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 whole plant of *Sarcostemma secamone* (150mg/kg body weight)

Group IV: Diabetic rats given ethanol extract of whole plant of *Sarcostemma secamone* (300mg/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 Otoluidine method[23]. Insulin level was assayed by Enzyme Linked Immuno Sorbant Assay (ELISA) kit[24]. Urea estimation was carried out by the method of Varley[25]; serum creatinine was estimated by the method of Owen *et al*[26]. Glycosylated haemoglobin (HBA1C) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan[27].

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

Serum protein[28] 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[29]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong[30].

Estimation of lipids and lipoprotein

Serum total cholesterol (TC)[31], total triglycerides (TG)[32], low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL- C)[33], high density lipoprotein cholesterol (HDL-C)[34] and phospholipids[35] 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.

RESULTS

The phytochemical screening of ethanol extract of *S.secamone* whole plant revealed the presence of alkaloid, catechin, coumarin, flavonoid, phenol, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein.

Acute toxicity study

The ethanol extract was safe upto a dose of 2000mg/kg body weight. Behavior of the animals was closely observed for the first 8 hr then at an interval of every 4hr during the next 48hr, the extract did not cause mortality on rats during 48hr observation or any behavioral change.

Body weight and fasting blood glucose (FBG) level changes in diabetic rats

In the present study, alloxan induced diabetic rats showed significant (p< 0.05) reduction in body weight (Table 1). The administration of *S.secamone* and glibenclamide to diabetic rats restored the changes in levels of body weight. Table 1 shows the dose dependent antihyperglycemic activity of *S.secamone* extracts. The FBG levels of diabetic rats were significantly (p<0.001) higher than those of normal control rats. When different doses of *S.secamone* were tested for their glucose lowering effects, the ethanol extract at a dose of 300mg/kg body weight produced the maximum fall in the FBG levels of diabetic rats after 2 week of treatment.

Table 1: Effect of whole plant extract of *Sarcostemma secamone* on the Body weight and Fasting Blood Glucose in Normal, Diabetic induced and Diabetic treated rats.

Parameter	Mean initial Body weight (g)	Mean final Body weight (g)	Mean weight Gain(G↑)/ Loss(L↓) (g)	Fasting Blood Glucose (mg/dl)	
				Initial	Final (after 2 wks)
Group I	201.56±8.32	219.35±6.42	17.81±0.39↑	78.36±2.17	74.53±1.51
Group II	194.11±8.32	170.14±5.84	23.97±1.34↓*	214.33±7.56***	201.74±3.13***
Group III	212.26±7.14	191.18±4.58	21.16±1.14↓	215.32±6.31	164.20±5.10
Group IV	214.54±6.34	194.34±4.86	20.20±1.34↓	215.64±7.80ns	123.31±6.34**b
Group V	198.34±5.36	191.59±5.13	6.75±0.34↓	193.98±3.46**	118.56±2.94**ab

Each Value is SEM of 5 animals: *p < 0.05 comparison with Normal Control vs Diabetic and Drug treated: * p < 0.05; **p <0.01; ***p <0.001; ns – Not significant a – p < 0.05 Diabetic Control vs Drug treated; b - p<0.05 comparison with initial vs final

Blood glucose and other parameters levels in diabetic rats

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<0.001) increase in blood glucose level in alloxan induced diabetic rats (Group II), when compared with normal rats (Group I). The administration of whole plant extract of *S.secamone* (Group III and IV) and glibenclamide (Group V) tends to bring the parameters (p<0.05) towards the normal. Serum insulin level of diabetic control group was significantly (p<0.001) decreased when compared to normal control group (Group I). The *S.secamone* whole plant extract and glibenclamide group of diabetic rats significantly (p<0.05; p<0.001) increased insulin. A significant elevation in urea and creatinine was observed in alloxan induced diabetic rats when compared to control rats. The *S.secamone* extracts were administrated orally to diabetic rats for 14 days reversed the urea and creatinine level to near normal. The administration of ethanol extract of *S.secamone* whole plant and glibenclamide (p<0.05; p<0.001) reduced HbA_1C.

Table 2: Effect of whole plant extract of *Sarcostemma secamone* on the Serum Insulin, Glucose, Urea, Creatinine and Glycosylated Haemoglobin level of Normal, Diabetic induced and Diabetic treated rats

Parameter	Insulin (mμ/ml)	Glucose (mg/dl	dl)	Creatinine (mg/dl)	Glycosylated Hb
Group I	18.59±1.53	76.99±1.26	10.53± 1.45	0.74±0.05	5.03±0.04
Group II	5.26±1.41***	201.56±14.33***	14.91±1.68*	0.88±0.02	9.36±1.16*
Group III	8.31±1.23[a]	184.06±4.93	13.14±1.38[a]	0.86±0.01	7.13±1.03[a]
Group IV	10.56±1.16a	139.53±1.94a	12.56±0.87	0.82±0.03	5.49±1.13
Group V	17.91±1.26aa	102.63±2.62aa	9.43±0.14a	0.79±0.05	4.97±0.84aa

Each Value is SEM of 5 animals: * p < 0.05; **p<0.01; ***p <0.001; *Comparison made between Normal Control and Diabetic Control and Drug treated groups : a - p<0.05; aa - p<0.01 Comparison made between Diabetic Control and Drug treated groups

Biochemical parameters levels in diabetic rats

The decreased total protein, albumin and globulin levels were noticed in diabetic control rats (Group II) compared to normal control rats (Group I) (Table 3). The administration of *S.secamone* whole plant extract 150 and 300mg/kg and glibenclamide significantly (p<0.05) increased total protein, albumin and globulin levels compared to diabetic control rats. Also, the SGPT, SGOT and ALP levels were elevated in alloxan induced diabetic rats compared to normal rats. Oral administration of *S.secamone* whole plant extract 300mg/kg and glibenclamide treatment reduced above parameters compared to diabetic control rats.

Table 3: Effect of whole plant extract of *Sarcostemma secamone* on the Serum protein, Albumin, Globulin, SGOT, SGPT and ALP levels of Normal, Diabetic induced and Diabetic treated rats.

Parameter	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group I	7.93±0.14	4.13±0.23	3.80±0.67	19.56±1.14	17.39±0.36	116.34±2.56
Group II	5.98±0.26*	3.48±0.14	2.50.±0.24	20.66±2.01	21.42±0.88	124.56±1.93
Group III	6.38±0.11	3.21±0.10	3.17±0.13	20.11±1.36	18.30±0.74	120.13±1.32
Group IV	7.84±0.14	4.04±0.46	3.80±0.71	19.33±0.93	16.39±0.84	119.56±1.84
Group V	8.24±0.19a	4.20±0.16	4.04±0.36a	17.56±0.84	18.54±0.91	108.51±1.88

Each value is SEM of 5 animals: * p < 0.05 *Comparison made between Normal Control and Diabetic Control and Drug treated groups: a: p<0.05 Comparison made between Diabetic Control and Drug treated groups

Lipid profiles

Table 4 shows the levels of TC, TG, LDL-C, VLDL-C, HDL-C and PL in the serum of diabetic rats showed significantly (p<0.05) increased serum lipid profiles except HDL-C when compared with normal rats. The ethanol extract of *S.secamone* whole plant treated rats showed a significantly (p<0.05) decreases 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. The administration of ethanol extract of *S.secamone* whole plant and glibenclamide to the diabetic rats; HDL-C level was found to be restored to normal.

Table 4: Effect of whole plant extract of *Sarcostemma secamone* on the Serum Lipid profile of Normal, Diabetic induced and Diabetic treated rats

Parameter	TC (mg/dl)	T G(mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	PL (mg/dl)
Group I	118.31±2.63	83.67±1.84	24.31±1.13	77.27±1.27	16.73±0.98	173.29±3.14
Group II	184.16±1.98**	116.26±1.80*	49.64±1.73	141.27±2.14**	13.25±1.03*	231.90±2.59*
Group III	156.74±1.34	104.28±1.30	32.16±1.56	121.10±1.10	18.16±1.12	212.42±2.76
Group IV	143.29±1.91	113.43±1.24	28.14±1.22	92.47±2.08	22.68±0.93	195.52±2.88
Group V	122.33±1.42a	98.26±0.94a	29.56±1.61a	73.12±1.83aa	19.65±0.78	176.87±3.04

Each Value is SEM of 5 animals: *p < 0.05; **p<0.01 *Comparison made between Normal Control and Diabetic Control and Drug treated groups: a - p<0.05; aa - p<0.01 Comparison made between diabetic control and drug treated groups

DISCUSSION

Commonly practiced pharmacologic treatment of diabetes mellitus includes oral hypoglycemic agents and insulin. There is an increasing demand by patients for the use of natural products and other dietary modulators with antidiabetic activity. This tendency is because insulin, to date, cannot be used orally and its repeated injections have many undesirable adverse effects. In addition, certain oral hypoglycemic agents are not effective in lowering the blood sugar in chronic diabetic patients. The global information on ethnobotanicals includes about 800 medicinal plants are used for controlling diabetes mellitus[36]. Dietary management includes the use of traditional medicines that are mainly derived from plants[37]. Even now, approximately 80% of the third world population is almost entirely dependent on traditional medicines. There are numerous traditional medicinal plants reported to have hypoglycemic properties[38-41].

The present study indicates the hypoglycemic and antihyperlipidaemic potential of *S.secamone* whole plant ethanol extract on alloxan induced diabetic rats. In the present study, induction of diabetes by alloxan, decreased in body weight. In diabetic rats, observed reduction in body weight was possible due to catabolism of fats and protein [42]. The administration of ethanol extract of *S.secamone* whole plant improves the body weight compared to diabetic control rats which indicates preventive effect of *S.secamone* whole plant extract on degradation of structural

proteins. Ethanol extract of *S.secamone* showed a dose dependent effect on FBG to a dose of 300mg/kg. Administration of alloxan led to more than 1.5 fold elevation of fasting glucose level which was maintained over a period of 2 weeks. Two weeks of daily treatment of *S.secamone* whole plant extract (300mg/kg) make fall in blood glucose level by 42.81%. The present findings indicate the hypoglycemic and potential antihyperglycemic nature of the extracts.

S.secamone whole plant ethanol extract (150 and 300mg/kg body weight) significantly (p<0.05) decreased blood glucose level and increase in serum insulin level in alloxan induced diabetic rats. Similarly ethanol extract of leaves of *Annona squamosa* (350mg/kg) showed reduction in blood glucose level was reported[43]. Similar antidiabetic activity was seen in the ethanol extract of leaves of *E.singampattina*[40]. Numerous mechanisms of actions have been proposed for these plant extracts. Some hypotheses relate to their effects on the activity of pancreatic β cells (synthesis, release, cell generation/ revitalization) or the increase in the protective /inhibitory effect against insulinase and the increase of the insulin sensitivity or the insulin like activity of the plant extracts. Other mechanisms may involve improved glucose homeostasis. All of these actions may be responsible for the reduction and or abolition of diabetic complications [44]. Hypoglycemic effects have been reported with other plants such as *Pithocellobium dulce*[45]; *Aloe vera*[46]; *Sphaeranthus indicus* [47]; *Waltakake volubilis*[48]; *Eugenia floccosa* [39]; *Polygala rosmarinifolia* [49] and *Anaphyllum wightii* [50] well known for their antidiabetic activities.

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 glycosylated protein tissue levels[51]. In the present study, significant increase in serum urea and creatinine levels was observed in diabetic rats compared to normal control rats which indicate impaired renal function in diabetic rats. The treatment with ethanol extract of *S.secamone* decreased the above parameters significantly (p<0.05) compared to diabetic control rats and it showed protective effect of ethanol extract of *S.secamone* on the kidneys.

Glycosylated haemoglobin is produced by glycosylation on haemoglobin. Glycosylated haemoglobin is formed progressively and irreversibly over a period of time and is stable over the life span of the red blood cells. It is unaffected by diet, insulin or exercise, even on the day of test. Therefore glycosylated haemoglobin can be used as an excellent marker of overall glycaemic control. Since it is formed slowly and does not dissociate easily, it reflects the real blood glucose level [52,53]. In this study, the diabetic rats had elevated levels of glycosylated haemoglobin and therefore, the significant decrease in the level of glycosylated haemoglobin in alloxan induced diabetic rats following *S.secamone* whole plant extract therapy indicates that the overall blood glucose level was controlled, probably due to improvement in insulin secretion[54]. It noteworthy that the serum insulin level in diabetic animals treated with *S.secamone* also increased when compared to the diabetic control animals. Thus, it seems that *S.secamone* whole plant stimulated increased insulin secretion in alloxan induced diabetic rats.

In diabetic condition, occurrence of reduction in protein and albumin may be due to proteinuria, albuminuria or increased protein catabolisms, which are clinical markers in diabetic nephropathy[55]. The protein and albumin level was reduced after the induction of diabetes and treatment of ethanol extract of whole plant *S.secamone* increased both levels considerably in diabetic rats towards normal level. This action possibly is through increase in the insulin mediated amino acid uptake, enhancement of protein synthesis and/or inhibition of protein degradation[56]. In the present study serum enzymes such as SGOT, SGPT and ALP were used in the evaluation of hepatic damage. In diabetic rats an increase in these enzyme activities reflects active liver damage. Increased levels of SGOT and SGPT under insulin deficiency[57] have been related with increased gluconeogenesis and ketogenesis during diabetes. Moreover, increased levels of these enzymes together with ALP are reported to be associated with liver dysfunction and leakage into the blood stream in diabetes [58]. Administration of *S.secamone* whole plant extract in diabetic rats resulted in reduction in the activities of these enzymes in serum compared to the diabetic control.

Diabetes mellitus is usually associated with prominent levels of serum lipids and such an increase causes the risk factor for coronary heart diseases. Alloxan induced diabetes also developed hyperlipidemia which is in agreement without previous observations[59,60]. In the present study, *S.secamone* whole plant extract significantly reduced the TC, TG, LDL-C and VLDL-C levels with an increase of HDL-C in treated diabetic rats compared to diabetic control rats. This may be due to the insullinotropic effect or insulin secretagogne activity of this extract.

In conclusion, this study has shown that the ethanol extract of *S.secamone* whole plant had hypoglycemic and hypolipidemic effects in alloxan induced diabetic rats. Several authors reported those secondary metabolites, such as saponins, flavonoids, phenolic compounds and triterpenoids have hypoglycemic and hypolipidemic activity[61,62]. Hence the hypoglycemic and hypolipidemic properties of *S.secamone*_may be due to different types of active secondary metabolites, each with a single or diverse range of biological activities. Further study need to be isolate, identify the active compounds and formation.

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