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HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF METHANOL EXTRACT OF *SAUROPUS BACCIFORMIS* WHOLE PLANT IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The methanol extract of *Sauropus bacciformis* (L.) Airy Shaw whole plant (Family:Euphorbiaceae) was investigated for it hypoglycemic and hypolipidemic effect in wistar albino rats. Diabetes was induced in albino rats by administration of alloxan monohydrate (150 mg/kg i.p). The Methanol extracts of *Sauropus bacciformis* at a dose of 150 and 300 mg/kg of body weight were administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of methanol extract of *Sauropus bacciformis* 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), and Serum glutamate oxaloacetate transaminases (SGOT), and alkaline phosphatase (ALP)] were measured in the diabetic rats. **In the acute toxicity study**, methanol extract of *Sauropus bacciformis* 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 methanol extract of *Sauropus bacciformis* whole plant compared to diabetic control rats. In diabetic rats, methanol extract of *Sauropus bacciformis* whole plant administration altered lipid profiles were reversed to near normal than diabetic control rats. From the above results, it is **conducted** that methanol extract of *Sauropus bacciformis* whole plant possess significant hypoglycemic and hypolipidemic effects in alloxan induced diabetic rats.

KEYWORDS: *Sauropus Bacciformis*, Hypoglycemic, Hypolipidemic, Alloxan, Glibenclamide.

INTRODUCTION

Medicinal plants are the most important source of life saving drugs and have been widely for the treatment of diseases in traditional way for several years. An interaction between ancient medicine and biotechnological tools is to be established towards newer drug development. The interface between cell biology, structural chemistry and in vitro assays will be the best way available to obtain valuable leads. The value of plants lies in the potential access to extremely complex molecular structure that would be difficult to synthesize in the laboratory. In spite of an increasing awareness and expenditure of resources, the incidence of chronic diseases like cardiac, cancer, diabetes etc has not declined and in fact is rising at an alarming rat (Dixit and Ali, 2010). Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. Diabetic mellitus is a metabolic disorder characterized by disturbances in carbohydrate, protein, lipid metabolism and by complications like microvascular (retinopathy,neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular diesase) complications. Currently available synthetic antidiabetic agents produce serious side effects like hypoglycemic coma and hepatorenal disturbences (Raja et al., 2017). Plants have played a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemic and hypolipidemic agents (Gandhi and Sasikumar, 2012).

Genus *Sauropus Blume* includes about 56 species. *S.androgynus* star (gooseberry) is cultivated in India for onsumption and commercial use. It is used as nutraceutical in health care and in prevention and treatment of diseases and it has anticancerous properties (Kanchanapoom et al., 2003; Jenecius et al., 2012). *Sauropus bacciformis* (L.) Airy Shaw is a herb growing in seashore sandy tracts, especially in brackish clayey soil near sea level to below 100 m. *Sauropus bacciformis* is commonly called Kuruvi Thengai, Thengai Keerai and it is used by the rural folk for medicinal purpose. The aerial parts of the plant is used against gastrointestinal problems. The plant paste is given with Piper betel orally

(Muralidharan and Narasimhan, 2012). The plant is also used by the local people for skin diseases. They use the aerial parts as a green vegetable. The main objective of this study was to assess the hypoglycemic and hypolipidemic effect of methanol extracts of *Sauropus bacciformis* in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant Material: The well grown whole plant of *Sauropus bacciformis* (L.) Airy Shaw was collected from coastal regions of Thoothukudi, District, Tamil Nadu. With the help of local flora, voucher specimens were identified and preserved in the Research Department of Botany, St.Mary's College, Tuticorin, Tamil Nadu for further references.

Preparation of plant extract for phytochemical screening and antidiabetic studies

The whole plant of *S.bacciformis*was shade dried at room temperature and dried whole plants were powdered in a Wiley mill.

Hundred grams of powdered S.bacciformis whole plant was packed in a Soxhlet apparatus and extracted with methanol. The extract was subjectedtoqualitative test for the identification of various phytochemical constituents as per the standard procedures [Vasantha *et al.*,2012; Murugan and Mohan, 2011]. The methanol extracts were concentrated in a rotary evaporator. The concentrated methanol extract were used for antidiabetic studies.

Animals

Normal healthy male Wistar albino rats (20-25gm) were housed under standard environmental conditions at temperature(25±20º C) and light and dark (12: 12 h). Rats were fed with standard pellet diet (Sai Durga Animal Feeds, Bangalore, 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 overnightand 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/kgbody 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]. Twodays after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200-260 mg/100ml 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 methanol extract of *Sauropus bacciformis* whole plant (150mg/kg body weight).

Group IV: Diabetic rats given methanol extract of *Sauropus bacciformis* whole plant (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 p eriod of 14 days by decapitation. Blood was collected, se ra 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 assayedby EnzymeLinked Immunosorbant Assay (ELISA) kit [Anderson*etal*.,1993]. Urea estimation was carried out by the method of Varley [1976]; serum creatinine wasestimated by themethod of Owen *etal.*, [1954]. Glycosylatedhaemoglobin (HBA1C) estimation was carried out by a modifiedcolorimetricmethod of Karunanayake and Chandrasekharan [1985].

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

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

Estimation of lipids and lipoprotein

Serum total cholesterol (TC)[ParekhJung,1970], total triglycerides (TG) [Rice,1970], low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL –C) [Friedwald and Levy,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.

RESULTS

Phytochemical constituents

The phytochemical screening of methanol extract of *S.bacciformis* whole plant revealed the presence of alkaloid, coumarin, flavonoid, phenols, saponins, steroids, tannins, terpenoids, sugar, and glycoside.

Acute toxicity study

The methanol extract was safe upto a dose of 2000 mg/kg body weight. Behavior of the animals was clearly observed for the first 8 hours then at an interval of every 4 hours during the next 48 hours, the extract did not cause mortality on rats during 48 hours 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 (Table1). The administration of *S.bacciformis* and glibenclamide to diabetic rats restored the changes in the levels of body weight. Table.1 shows the dose dependent antihyperglycemic activity of *S.bacciformis*extracts.The FBG levels of diabetic ratswere significantly (p<0.001) higher than those of normal control rats.When different doses of *S.bacciformis* were tested for their glucose lowering effects, the methanol extract at a dosage of 300 mg/kg body weight produced the maximum fall in the FBG levels of diabetic rats after 2 weeks of treatment.

Table. 1: Effect of methanol extracts of *Sauropus bacciformis* whole plant on the Body weight and Fasting Blood Glucose in Normal, Diabetic induced and diabetic treated rats.

Parameter	Mean initial Body Weight(gm)	Mean final Body Weight(gm)	Mean weight Gain(G↑)/Loss(L↓)g	Fasting Blood Glucose(mg/dl) Initial	Final(after 2 wks)
Group I	213.56±9.42	218.06±8.36	5.09↑	68.34±1.31	78.65±6.54
Group II	226.83±8.16	204.54±9.89	22.29↓**	221.66±11.82***	214.59±9.64***
Group III	216.53±9.84	219.16±5.86	2.63↑	206.13±10.84***	152.16±8.28*^{ab}
Group IV	211.56±7.65	218.16±8.27	6.60↑	193.58±8.25***	129.46±7.65^{aabb}
Group V	208.16±9.27	214.28±9.66	6.12↑	198.16±7.58***	108.31±5.66^{aabb}

Each Value is SEM of 5 animal:* 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 intial vs final.

Blood glucose and the other parameters levels of 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.bacciformis*(GroupIII and IV) and glibenclamide (GroupV) 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 controlgroup (GroupI).The plant extract and glibenclamide group of diabetic rats significantly (p<0.05;p<0.01)increased insulin.A significantly elevation in urea and creatinine was observed in alloxan induced diabetic rats when compared to control rats. The *S.bacciformis* extracts were administered orally to diabetic rats for 14 days reversed the urea and creatinine level to near normal.The *S.bacciformis* whole plant and glibenclamide(p<0.05; p<0.001) reduced HbA1C.

Table. 2: Effect of methanol extracts of *Sauropus bacciformis* whole plant 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)	Urea (mg/dl)	Creatinine (mg/dl	Glycosylated Hb
Group I	16.23±1.94	81.23±1.64	13.28±0.67	0.63±0.04	4.34±0.03
Group II	7.84±1.31**	224.88±8.93***	24.67±1.22*	2.98±0.07**	11.84±1.23**
Group III	8.27±1.93*	159.65±7.34^{**aa}	19.66±0.38*	2.88±0.05**	11.04±0.36*
Group IV	12.88±2.04^{nsa}	127.68±14.16^{*aa}	16.28±0.45^{a}	1.16±0.03^{a}	6.63±0.21^{aa}
Group V	15.27±1.93^{nsa}	93.54±3.54^{aaa}	15.18±0.78^{a}	0.93±0.07^{aa}	5.81±.013^{aa}

Each Value is SEM of 5 animal:* p<0.05p<0.001. Comparison made between Normal Control and Diabetic control and Drug treated group. ^{a}p<0.05;^{aa} -p<0.01- Comparison made between Diabetic Control and Drug treated group.

Biochemical parameters levels in diabetic rats

The decreased total protein, albumin and globulin levels were noticed in diabetic control rats (GroupII) (Table3).The administration of *S.bacciformis*whole plant extract 150 and 300 mg/kg and glibenclamide significant (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 control rats. Oral administration of *S.bacciformis* whole plant extract 300

mg/kg and glibenclamide treatment reduced above parameters compare to diabetic control rat.

Table 3: Effect of methanol extracts of *Sauropus bacciformis* whole plant on the Serum protein, Albumin, Globulin, SGOT, SGPT and ALP level of Normal, Diabetic induced and diabetic treated rats.

Parameter	Protein(g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (µ/l)	SGOT(µ/l)	ALP(µ/l)
Group I	9.16±1.13	4.83±0.14	4.28±0.31	13.22±0.67	19.36±0.13	213.16±6.93
Group II	6.04±0.81**	4.16±0.24	1.88±0.65**	31.62±1.39**	34.22±0.98*	263.93±11.96*
Group III	7.88±0.22	4.42±0.27	3.46±0.32ns	21.63±1.02	20.62±1.13	228.16±9.16
Group IV	8.13±0.84a	4.28±0.64	3.85±0.14a	18.17±0.93	19.97±0.84	220.62±8.33
Group V	8.68±0.16a	5.08±0.39a	3.60±0.16a	15.93±0.81	17.16±0.17a	194.51±6.73a

Each Value is SEM of 5 animal. * p<0.05. Comparison made between Normal Control and Diabetic control and Drug treated group. a p <0.05; Comparison made between Diabetic Control and Drug treated group.

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 methanol extract of *S.bacciformis* whole plant treated rats showed a significant (*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. The administration of methanol extract of *S.bacciformis* whole plant and glibenclamide to the diabetic rats, HDL-C level found to be restored to normal.

Table. 4: Effect of methanol extracts of *Sauropus bacciformis* whole plant on the Serum Lipid profile of Normal, Diabetic induced and diabetic treated rats.

Parameter	TC(mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	VLDL (mµ/dl)	HDL(mµ/dl)	PL(mµ/dl)
Group I	128.55±4.83	112.84±2.93	34.63±1.21	22.56±1.34	71.36±2.16	182.40±3.54
Group II	184.65±5.95***	166.28±2.15**	21.84±1.05*	33.25±1.87*	129.56±2.87**	232.33±4.76**
Group III	158.28±2.91*	149.68±2.65a	24.92±1.34*	29.93±1.42*	103.43±1.36*	208.86±2.16a
Group IV	139.38±2.45nsa	118.51±1.93	32.86±1.96aa	23.70±1.51a	89.82±1.57a	198.27±1.32a
Group V	133.94±3.29aa	124.63±2.56	31.14±1.24aa	24.92±1.23a	77.88±1.48a	187.20±1.47aa

Each Value is SEM of 5 animal. * p<0.05; ** p<0.01 Comparison made between Normal Control and Diabetic control and Drug treated group. a p <0.05; aa p<0.01- Comparison made between Diabetic Control and Drug treated group.

DISCUSSION

Diabetes mellitus patients in India are increasing day by day probably due to change in lifestyle, change in food pattern i.e. from traditional fiber rich diet to sugary fast food diet and also because of genetic basis. The disorder being chronic in nature needs long term treatment to prevent the complications arising due to persistent high blood glucose level (Tiwari and Madhusudana, 2002). Pharmacotherapy available for the treatment of diabetes in modern healthcare system includes insulin and oral hypoglycemic drugs (Tripathi, 2003). However due to economic constraints, it is not possible for majority of the diabetic patients in developing countries like India to use these drugs on regular basis. Moreover these synthetic antidiabetic drugs are associated with large number of adverse effects. Hence there is increase in the trend to use traditional indigenous plants widely available in India for the treatment of diabetes mellitus. Over 150 plant extract and some of their active principles including flavonoids, tannins, alkaloids etc are used for the treatment of diabetes (Erememisoglu et al., 1995). However very few of these plants have been screened pharmacologically (Grover et al., 2002).

Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas.Alloxan causes a massive reduction in insulin release by the destruction of β-cells of the islets of langherhans, thereby inducing hyperglycemia. Insulin deficiency leads to various metabolic alterations in the animals *viz.* increased cholesterol, increased levels of alkaline phosphate and transaminases (Sharma et al., 2010; Bhatt et al., 2009).

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 increase in blood glucose level, decrease in body weight and polyuria. In diabetic rats, observed reduction in body weight was possible due to catabolism of fats and protein (Veeramani et al., 2008).The administration of methanol extract of *S.bacciformis* improves body weight compared to diabetic control rats which indicates preventive effect of *S.bacciformis* 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

methanol extract of *S.bacciformis* 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 (Sezik et al., 2005). Earlier, many plants have been studied for their hypoglycemic and insulin release stimulatory effects (Pattabiraman and Muthukumaran, 2011; Maruthupandian and Mohan, 2011; Shanmugasundaram et al., 2011; Kala et al., 2012; Kala et al., 2012;Shajeela et al., 2012;Sheela and Uthayakumari, 2017).

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 (Lal,2009). 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 methanol extract of *S.bacciformis* lowered the above parameters significantly compared to diabetic control rats and it showed protective effect of methanol extract of *S.bacciformis* on the kidneys.

In diabetes, HbA1c 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(Deguchi, and Miyazaki, 2010),(Lanjhiyana, 2011). HbA1c 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 (Alagammal, 2012). 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 methanol extract of *S.bacciformis* 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.

In diabetic condition, occurrence of reduction in protein and albumin may be due to proteinuria, albuminuria or increased protein catabolism, which are clinical markers in diabetic nephropathy (Kaleem, 2008). The protein and albumin level was reduced after the induction of diabetes and treatment of methanol extract of *S.bacciformis* 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 (Ramachandran et al., 2012). Also, increased serum SGOT, SGPT and ALP levels were reported in diabetes and it may be due to liver dysfunction (Rao et al., 1989). In this study, increased level of SGOT, SGPT and ALP was observed in alloxan-induced diabetic rats which may have occurred by leakage of enzymes from the liver cytosol into the blood stream; it represents the toxicity of alloxan on liver. Diabetic rats treated with methanol extract of *S.bacciformis* significantly reduced both enzyme levels which represents the protective action of methanol extract of *S.bacciformis* in diabetic condition.

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Al-Shamaony et al.,2009). The abnormal high concentrations of serum lipids in diabetic animals are mainly due to an increased mobilization of free fatty acids from peripheral fat depots (Al-Logmani and Zari, 2009). In the present study, significantly increased levels of serum TC, TG, V-LDL and LDL as well as marked reduction in serum HDL level in diabetic rats. Administration of both the doses of methanol extract of *S.bacciformis* decreased levels of TC, LDL, V-LDL and TG levels as well as increased the level of HDL in diabetic rats. The above action could be beneficial in preventing diabetic complications such as coronary heart diseases and atherosclerosis in diabetic condition. Increased phospholipids levels in serum were reported by Anitha *et al*. in alloxan induced diabetic rats (Anitha et al., 2012). Administration of methanol extract of *S.bacciformis* glibenclamide decreased the levels of phospholipids.

In the present study, the administration of *S.bacciformis* methanol extracts to alloxan induced hyperglycemic rats demonstrated prominent reduction in blood sugar level, normalization of serum biochemical profile including lipid content, as compared to alloxan control rats. The phytochemical analysis has shown the presence of potent phytochemicals such as flavonoids, terpenoids, glycosides, steroids, saponin and phenols. Several authors reported that flavonoids, steroids,terpenoids, phenolic acids are known to be bioactive antidiabetic principles(Alagammal et al., 2012), (Anitha et al., 2012). Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secretagogues (Alagammal et al., 2012). In the present study, the phytochemical analysis of methanol extract of *S.bacciformis* clearly prints out the presence of above said active principles. The preliminary investigation on the antihyperglycemic, antihyperlipidaemic and antioxidant efficacy of methanol extract of *S.bacciformis* will be significant to proceed further in this path for the isolation of active principles responsible for the antidiabetic activity.

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