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Antidiabetic Effect of *Sauropus androgynus* L. Leaves in Alloxan Induced Diabetic Mice

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Green leafy vegetables are efficient ameliorator of oxidative stress associated with diabetes mellitus (DM). The aim of the present investigation was to evaluate the antidiabetic activity of *Sauropus androgynus* leaf extract (SALE) in alloxan induced diabetic mice. Oral administration of *Sauropus androgynus* leaf methanol extract (250mg/kg and 500 mg/kg) was studied in normal and alloxan induced diabetic mice for fifteen days. The parameters studied included oral glucose tolerance test (OGTT), fasting blood glucose (FBG), serum lipid profile, liver function test, antioxidant enzymes and liver glycogen content. In OGTT reduction of blood glucose levels took place from 60 min of extract administration. The extracts produced a dose dependent fall in FBG. After treatment with SALE there was significant reduction in the levels of serum lipid profile and liver function test level, decrease in lipid peroxidation (TBARS) and increase in superoxide dismutase (SOD), catalase (CAT) and stimulated the glutathione (GSH) production in the liver and significant increase in the liver glycogen content was observed. The results demonstrate that SALE possesses significant antidiabetic activity in diabetic mice. The results suggest that SALE administration could be used as antidiabetic constituent in case of DM. This may be related to its anti-oxidative properties.

Key words: *Sauropus androgynus* leaves, Antidiabetic, Alloxan, Glucose, Lipids.

Diabetes is a chronic disease that occurs when the body cannot produce enough insulin or cannot use insulin effectively. This leads to an increased concentration of glucose in the blood causing a condition known as hyperglycemia together with disturbances of carbohydrate, fat and protein metabolism resulting from defects of insulin secretion, insulin action or both[1]. Insulin is a hormone produced in the pancreas that allows glucose from food to enter the body's cells where it is converted into energy needed by muscles and tissues to function. The disease causes abnormalities in the metabolism of lipid and protein[2]. Different extracellular proteins are also modified into glycoprotein due to high blood glucose, which is associated with severe diabetic complications[3]. Over time the raised blood sugar or diabetes is also associated with generation of reactive oxygen species (ROS) and consequent oxidative damages particularly in liver, kidney and pancreas[4]. ROS are also involved in the progression of insulin resistance as well as pancreatic â-cell dysfunction[5]. Vitamins C and E are the natural antioxidants have been reported to decrease the oxidative stress in experimental diabetes[6].

The plants use different mechanisms for reducing blood sugar levels, which reveal properties similar to that of well-known sulfonylurea drugs like glibenclamide, which cures hypoglycemia in normal animals by stimulating insulin release from pancreatic â-cells, besides reducing hepatic clearance of insulin hormone[7, 8]. World Health Organization (WHO) has recommended the evaluation of traditional plant

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treatments for diabetes as they are effective, non-toxic, with less or no side effects and are considered to be excellent candidates for oral therapy[9]. *Sauropus androgynus* L., belonging to the family Euphorbiaceae and it is a perennial shrub, cultivated in India, Sri Lanka, Thailand, Laos, Malaysia, Indonesia and Southeast Asia. In India found growing in Sikkim, Khasi Hills, Western ghats and south India. The exact origin of *Sauropus* is unknown. It is commonly known as star goose berry (Tropical asparagus, Chekkurmanis), multivitamin, multigreen plant. In Tamil known as Thavasai murungai and in Malayalam Madhuracheera. *Sauropus* is highly nutritious for its substantial vitamin content[10]. Decoction is given in stricture of the bladder and in fevers also used as diuretic. Fresh leaves are an excellent source of provitamin A, Carotenoids, vitamins B and C, protein and minerals. Mature leaves have more nutrients than young leaves. Vitamin C content was high in raw and cooked *Sauropus androgynus*[11]. SA has gained popularity as a weight reducing vegetable[12].

MATERIALS AND METHODS

The leaves of the plant *Sauropus androgynus* (SA) were collected from Maruthancode, Kanya Kumari district (TamilNadu, India). The plant specimens were further authenticated at the Botanical survey of India Coimbatore, voucher specimens of the sample (BSI/SRC/5/23/2011-12/Tech/1129) have been deposited in the Herbarium of the department. The Institutional Animal ethics committee, School of biotechnology and health sciences (IAEC/KU/BT/12/020) approved the study.

Alloxan monohydrate and Chloroform were purchased from sigma Chemicals Co. Mice were randomly divided in to the following five experimental groups (each group contained six mice) Group I (control group) mice received only basal diet without any treatment. In Group II Diabetic control mice, diabetes was induced by alloxan (150 mg/kg i.p.) and received only tween 80, 5% v/v in normal saline. Group III Diabetic mice received glibenclamide 2.5 mg/kg as standard drug, Group IV Diabetic mice administered S.A. extract 250mg/kg Group V Diabetic mice administered S.A. extract 500mg/kg for 15 days.

Antidiabetic activity of SALE was assessed in normal, glucose loaded and alloxan induced diabetic mice. OGTT was determined as previously described[13]. The FBG was estimated on days 0, 5, 10, and 15 of extracts administration. At the end of the experimental period, the concentration of serum lipid profiles such as triglycerides (TG), total cholesterol (TC), High-density lipoprotein (HDL) levels were determined by enzymatic methods and using commercial kits and liver function test such as Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) and Alkaline phosphatase (ALP) were done using Kits obtained from Span Diagnostics, India. Lipid peroxidation in liver was estimated by measuring thiobarbituric acid reactive substances (TBARS) and the activity of superoxide dismutase (SOD) activity and catalase (CAT) and the Glutathione (GSH) content in liver were determined as described[14 - 17]. The liver glycogen levels were estimated using anthrone reagent[18]. Protein content in tissue homogenate was measured by the method of Lowry[19]. Statistical analysis of the results was performed using one way ANOVA followed by Dunnet test using STAT software. The values were considered significant when $p<0.05$.

RESULTS

In oral glucose tolerance test the normal mice treated with glibenclamide (2.5 mg/kg; p.o) and mice treated with SALE 500 mg/kg; p.o showed a marked reduction in blood glucose level showing maximum effect at 30-120 min and 60-120 min with a significant level of ($p<0.01$) compared with control group. The mice treated with SALE 250 mg/kg; P.o also reduced the blood glucose level ($p<0.05$) at 60-120 min intervals compared to control group, however reduced the blood glucose level and the effect was found to be significant Figure 1. The diabetic control group produced a significant elevation in blood glucose concentration. Administration of extracts to diabetic mice showed a significant decrease in glucose level as compared with alloxan diabetic mice Table 1. More over the alloxan injected mice showed significant increase in the serum lipid levels, triglycerides and total cholesterol with a significant decrease in serum HDL level. Low density lipoprotein (LDL) is

considered toxic to the body and was significantly raised in comparison to that of the diabetic control group. SALE dose dependently ameliorated the high level of LDL with significant difference in comparison to that of diabetic control group. A decrease in very low density lipid (VLDL) levels were observed in Table 2. The SALE extract at both the doses significantly decreased the SGOT and SGPT levels respectively ($p<0.01$) and there was a significant increase in ALP level in alloxan induced diabetic mice in comparison to that of the normal control mice Table 3.

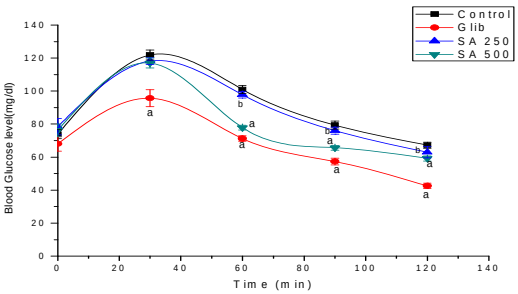

Fig. 1. Effect of SALE on OGTT on mice. Values are mean ± SD of 6 animals. Where a=$p<0.01$ and b=$p<0.05$ compared to control. (Abbreviation: SA- Sauropus androgynus; Glib-Glibenclamide).

A significant decrease in liver glycogen content was observed in diabetic mice compared to normal control group. Mice treated with SALE 250 mg/kg; p.o. did not show any significant increase in liver glycogen level, whereas treatment with glibenclamide and SALE 500 mg/kg; p.o. showed significant increase in liver glycogen content when compared to diabetic control mice. Effect of SALE on hepatic antioxidant enzymes TBARS, SOD, CAT, and GSH content is shown in Table 4. The protein level was significantly decreased in diabetic animal group. The protein level was significantly restored by SALE administration with 500 mg/kg and glibenclamide in comparison to that of diabetic control. Similarly,

Table 1. Effect of SALE on serum lipid level in alloxan induced diabetic mice

Group	Blood glucose (mg/dL) 0th day Mean±SD	Blood glucose (mg/dL) 5th day Mean±SD	Blood glucose (mg/dL) 10th day Mean±SD	Blood glucose (mg/dL) 15th day Mean±SD
Normal control	73.92±2.79	121.90±2.96	101.01±2.43	79.41±2.32
Diabetic control	164.80±1.24	204.33±4.06	199.98±3.00	196.60±2.54
Diabetic + glibenclamide	158.64±2.68*	136.00±3.98**	120.23±2.90**	106.69±2.31**
Diabetic + SALE 250 mg/kg	162.17±5.54 ns	198.70±4.82ns	152.17±6.13**	137.17±2.66**
Diabetic + SALE 500 mg/kg	160.97±4.07 ns	139.80±3.03**	125.55±5.02**	117.63±2.82**

All values are mean ± SD of 6 animals.
* Significant ($p<0.05$) when compared with the diabetic group.
* Significant ($p<0.01$) when compared with the diabetic group.

Table 2. Effect of SALE on serum lipid level in alloxan induced diabetic mice.

Group	Triglycerides (mg/dL) Mean±SD	Total cholesterol (mg/dL) Mean±SD	HDL (mg/dL) Mean±SD	LDL (mg/dL) Mean±SD	VLDL (mg/dL) Mean±SD
Normal control	104.16±2.48	88±3.16	64.5±3.93	2.83±2.31	20.83±0.40
Diabetic control	215.66±9.09	165±4.14	51.5±1.37	70.33±4.92	43.16±1.94
Diabetic + glibenclamide	124.33±3.14**	89±1.41**	38.5±2.07**	25.5±2.25**	24.83±0.75**
Diabetic + SALE 250 mg/kg	178.83±4.95**	114.66±4.08**	47.16±1.16**	31.66±5.88**	35.66±1.03**
Diabetic + SALE 500 mg/kg	144.83±3.06**	96.83±2.31**	57±1.78**	11±3.68**	28.33±0.75**

All values are mean ± SD of 6 animals.
* Significant ($p<0.05$) when compared with the diabetic group.
* Significant ($p<0.01$) when compared with the diabetic group.

there was decrease in lipid peroxidation (TBARS) and increase in SOD, CAT and stimulated GSH content when compared to that of diabetic control group. The hepatic GSH content was increased significantly in SALE treated group over the diabetic control group. However, SALE high dose treated group showed significant difference with glibenclamide treated group in comparison to diabetic control group.

DISCUSSION

Diabetes mellitus is a major health issue influencing major population worldwide and is widely recognized as one of the leading causes of death and disability. The limitations of currently available antidiabetic agents for control of blood glucose have stimulated research on novel antidiabetic agents with different mechanism of action[20]. The active principles from plant sources might act by several mechanisms such as stimulating insulin secretion, increasing repair/ proliferation of â cells, enhancing the effect of insulin and adrenaline and increasing the antioxidative properties[21]. The present results demonstrated the SALE significantly ameliorated the adverse influence of alloxan. The extracts showed a dose dependent fall in fasting blood glucose in alloxan induced diabetic mice. Alloxan induces diabetes by pancreatic beta cell damage mediated through generation of cytotoxic oxygen free radicals[22]. Alloxan is known for its chemical induction of diabetes in a wide variety of animal species by damaging the insulin secreting cells of the pancreas. Due to this a large number of â cells get damaged and there will be decrease in endogenous insulin release, and this leads to decreased use of glucose by the tissues[23].

The serum lipid profile is often increased in diabetes mellitus and this increase in lipid level cause an increased risk in coronary heart disease[24]. In our experimental model of DM, it was observed that alloxan administration produced a significant increase in serum lipid level and the treatment with SALE resulted in improved lipid profile. The decrease in hepatic glycogen content in diabetes has been observed in earlier studies[25]. And in this study it is probably due to lack of insulin in the diabetic state which results in the inactivation of glycogen synthase system. The hepatic glycogen content also significantly increased in SALE treated mice compared with diabetic mice. Liver is the main organ for metabolism, detoxification, storage and excretion of xenobiotics and their metabolites. SGOT, SGPT and ALP are trustworthy markers of liver function[26]. The results from the present study also indicates that SALE may reduce the level of liver function test. Diabetes is a chronic sickness, which produces oxidative stress. Liver antioxidant enzymes TBARS, SOD, CAT, GSH were recovered with SALE treatment in the liver Tissue[27]. A stimulating effect of the synthesis GSH by SALE was seen in the present study. The GSH reacts with free radicals and produces a crucial substrate for glutathione peroxidase and glutathione-S-transferase which takes place in the cellular defense mechanisms against intermediate oxygen products[28]. The ameliorative effect of SALE on hepatic lipid peroxidation produced by alloxan may be related to the significant rise in hepatic GSH induced by the active components in the

Table 3. Effect of SALE on liver serum markers level in alloxan induced diabetic mice

Group	SGOT AST IU/L Mean±SD	SGPT ALT IU/L Mean±SD	Alkaline phosphatase IU/L Mean±SD
Normal control	24.45±3.04	22.1±1.85	49.72±2.21
Diabetic control	55.39±2.41	46.26±2.60	113.45±3.17
Diabetic + glibenclamide	28.28±2.50 **	26.22±2.06 **	61.02±2.84 **
Diabetic + Methanol extract 250 mg/kg	44.78±3.09 **	35.65±2.60 **	73.22±2.42 **
Diabetic + Methanol extract 500 mg/kg	34.77±2.89 **	30.94±1.85 **	66.44±2.84 **

All values are mean ± SD of 6 animals.
* Significant ($p<0.05$) when compared with the diabetic group.
* Significant ($p<0.01$) when compared with the diabetic group.

Table 4. Effect of SALE on liver protein TBARS, SOD, CAT, GSH content in alloxan induced diabetic mice.

Group	Protein (µg/mg tissue) Mean±SD	TBARS (nM/min/mg protein) Mean±SD	SOD (U/mg protein) Mean±SD	CAT (U/min) Mean±SD	GSH (µM/g tissue) Mean±SD	Liver glycogen (mg/g)
Normal control	6.9±0.35	2.3±0.41	11.06±0.81	9.38±0.58	50.2±3.66	20.81±1.18
Diabetic control	2.66±0.70	6.41±0.53	4.41±0.61	3.36±0.60	22.6±3.97	9.69±0.56
Diabetic + glibenclamide	5.61±0.45 **	3±0.33 **	9.43±0.62 **	7.81±0.82 **	45.7±7.65 **	15.41±0.97**
Diabetic + Methanol extract 250 mg/kg	3.41±0.39 ns	5.65±0.37 *	5.5±0.56 *	5.55±0.52 **	30.88±4.21 *	10.79±0.53ns
Diabetic + Methanol extract 500 mg/kg	5.9±0.31 **	3.26±0.56 **	10.38±0.73 **	7.41±0.64 **	45.08±5.19 **	12.72±0.48**

All values are mean ± SD of 6 animals.
* Significant ($p<0.05$) when compared with the diabetic group.
* Significant ($p<0.01$) when compared with the diabetic group

plant leaf extract. SOD activity was also increased in mice administered with SALE as compared with diabetic control. SOD is responsible for removal of superoxide radicals[29]. Administration of SALE restored antioxidant enzymes to standard level in the liver of diabetic mice.

The data from our study conclude that SALE possesses significant antidiabetic activity and it may prove to be effective for the treatment of diabetes mellitus. However longer duration studies are required to show the exact mechanism of action so as to develop it as a potent antidiabetic drug.

REFERENCES

1. World Health Organization (WHO): Report of a WHO consultation. Definition, diagnosis and classification of diabetes mellitus, WHO/NCD/NCS/99.2. Geneva, WHO, 1999.
2. Kuzuya T, Nakagawa S, Satoh J, Kanazawa Y, Iwamoto Y and Kobayashi M: Report of the committee on the classification and diagnostic criteria of diabetes mellitus, *Diabet. Res. Clin. Prac* 2002; **55**: 65-85.
3. Glugliano D, Ceriello A, Paolisso G: Oxidative stress and diabetic vascular complications. *Diabetes Care* 1996; **19**: 257-267.
4. Baynes JW, Thorpe SR: Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. *Diabetes* 1999; **48**: 1-9.
5. Evans JL, Goldfine ID, Maddux BA, Grodsky GM: Oxidative stress and stress activated signalling pathways: A unifying hypothesis of type 2 diabetes. *Endocr Rev.* 2002; **23**: 599-622.
6. Garg MC, Bansal DD: Protective antioxidant effect of vitamins C and E in streptozotocin induced diabetes rats. *Indian J Exp Biol*. 2000; **38**: 101-104.
7. Ivorra MD, Paya M, Villar A: Hypoglycemic and insulin release effects of tormentic acid, a new hypoglycemic natural product. *Planta Medica* 1988; **54**: 282-286.
8. Davis SN, Granner DK: Insulin, oral hypoglycemic agents and the pharmacology of the endocrine pancreas. In: Goodman LS, Gilman AG: (Eds), the Pharmacological Basis of Therapeutics, 9th Ed. Mcgraw Hill, New York 1996; pp. 1487-1511.

9. Day C: Traditional plant treatments for diabetes mellitus: pharmaceutical foods. *Br.J. Nutr.* 1998; **80**: 203-208.
10. Padmavathi P and Prabhakara Rao M: Nutritive value of Sauropus androgynus leaves. *Plant Foods for Human Nutrition* 1990; **40**: 107-113.
11. Weenanan Somsub, Ratchanee Kongkachuichai, Pongtorn Sungpuag, Rin Charoensiri, Effects of three conventional cooking methods on vitamin C, tannin, myo-inositol phosphates contents in selected Thai vegetables. *Journal of food composition and analysis* 2008; **21**: 187-197.
12. Chen IC, Chang KC, Hsieh YK, Wu D: Torsade de pointes due to consumption of *Sauropus androgynus* as a weight reducing vegetable. *American Journal of Cardiology* 1996; **78**: 1186-1187.
13. Syiem D, Syngai G, Khup PZ, Khongwir BS, Kharbuli B, Kayang H: Hypoglycemic effects of *Potentilla fulgens L.* in normal and alloxan induced diabetic mice. *J Ethnopharmacol* 2002; **83**: 55-61.
14. Khan RA, Khan MR, Sahreen S, Ahmed M, and Shah NA: Carbon tetrachloride induced lipid peroxidation and hyperglycemia in rat: a novel study, Toxicology and Industrial Health. 2013.
15. Kakkar P, Das B, Viswanathan PN: A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys*, 1984; **21**: 130-132.
16. Sinha AK: Colorimetric assay of catalase. *Anal Biochem.* 1972; **47**: 389-394.
17. Shah NA, Khan MR, Ahmad B, Noureen F, Rashid U and Khan RA: Investigation on flavonoid composition and anti-free radical potential of *Sida cordata*, *BMC Complementary and Alternative Medicine* 2013; **13** (1); p. 276.
18. Van der vies J: Two methods for the determination of glycogen in liver. *J Biol Chem.*1953; **57**: 410-416.
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with folinphenol reagent. *J Biol Chem.* 1951; **193**: 265-275.
20. Reddy VS, Sahay RK, Bhadada SK, Agrawal JK, Agrawal NK: Newer oral antidiabetic agents. *Journal Indian Academy of Clinical Medicine* 2000; **1**: 245-251.
21. Shanmugasundaram ER, Gopinath KL, Radha SK, Rajendran VM, Possible regeneration of the islets of Langerhans in streptozotocin diabetic rats given Gymnema sylvestra leaf extracts. *J Ethnopharmacol* 1990; **30**: 265-279.
22. Shankar MB, Parikh JR, Geetha M, Mehta RS, Saluja AK, Antidiabetic activity of novel androstane derivatives from Syzygium cuminii Linn. *Journal of natural Remedies* 2007; **7**: 214-219.
23. Lenzen S: The mechanisms of alloxan and streptozotocin induced diabetes. *Diabetologia* 2008; **51**: 216-226.
24. Betteridge J: Lipid disorders in diabetes mellitus; in Pickup JC, Williams G. (eds) Text book of diabetes. 2nd edition. London. Blackwell Science 1997; 55.1-55.31.
25. Whitton PD, Hems DA: Glycogen synthesis in perfused liver of streptozotocin diabetic rats. *Biochemical Journal* 1975; 150, 153.
26. Ohaeri OC: Effect of garlic oil on the levels of various enzymes in the serum and tissues of streptozotocin diabetic rats. *Bioscience Reports* 2001; **21**: 19-24.
27. Kumari K and Augusti KT: Antidiabetic and antioxidant effects of S-methyl cysteine sulfoxide isolated from onions (Allium cepa. Linn) as compared to standard drugs in alloxan diabetic rats, *Indian Journal of Experimental Biology* 2002; **40**(9); pp. 1005-1009.
28. Protective Effect of Ethanol Extracts of Hericium erinaceus on Alloxan-Induced Diabetic Neuropathic Pain in Rats. Yi Z, Shao-Long Y, Ai-Hong W, Zhi-Chun S, Ya-Fen Z, Ye-Ting X, Yu-Ling H. *Evid Based Complement Alternat Med*. 2015;2015:595480. doi: 10.1155/2015/595480. Epub 2015 Apr 16.
29. Grape Seed Proanthocyanidin Extract Alleviates Arsenic-induced Oxidative Reproductive Toxicity in Male Mice. Li SG, Ding YS, Niu Q, Xu SZ, Pang LJ, Ma RL, Jing MX, Feng GL, Liu JM, Guo SX. *Biomed Environ Sci.* 2015 Apr;**28**(4):272-80. doi: 10.3967/bes2015.038.