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ANTI-DIABETIC POTENTIAL OF METHANOLIC EXTRACT OF LEAVES OF *SIDA SPINOSA* ON HIGH FAT DIET FED AND LOW DOSE STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ARTICLE IN

Key Words

Sida spinosa, Streptozotocin, diabetes and glibenclamide.

ABSTRACT

Objective: The aim of the present investigation was to evaluate the anti-diabetic potential of methanolic extract of *Sida spinosa* on high fat diet and low dose streptozotocin (HFD+STZ) induced diabetes in rats. Diabetes was induced by intraperitoneal injection of streptozotocin (150 mg/kg). **Methods:** Normal and diabetic rats were divided into different groups (n=6) and orally administered with methanolic extract (200mg/kg) and glibenclamide (5mg/kg) for 14days. At end of the experiment the blood samples were collected from experimental animals for biochemical estimation and the animals were sacrificed by cervical dislocation under mild anaesthesia. **Results and Discussion:** Significant reduction in blood glucose levels were observed in methanol extract of *Sida spinosa* (at the dose of 200 and 400 mg/kg) and glibenclmide (5 mg/kg) than that of diabetic rats. **Conclusion:** These results demonstrate the antidiabetic potential of *Sida spinosa* suggests that the plant may have potential therapeutic value in diabetes and related complications. Furthermore, experiments at the clinical levels are required to confirm the utility of this plant by traditional practitioners in the management of diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by deregulation of glucose and lipid metabolism. The global incidence of type 2 diabetes is projected to double to 350 million cases by the year 2030. Regions with greatest potential are Asia and Sub-Saharan Africa, where diabetes mellitus rates could rise to two or three folds than the present rates. Many drugs have been used in the prevention and treatment of DM. However, it has been reported that treatment with the synthetic drugs is responsible for various side effects and poor bioavailability. Hence, studying the medicinal value of plants and its active compound is always welcomed for the global benefit. Discovery of a single new synthetic drug would take about 10–15 years of time with a budget of 100–300 million US$. *Sida spinosa* Linn. (Malvaceae) is an erect, branched small perennial herb (or) small shrub which grows abundantly on cultivated fields, waste areas, road sides and open clearing in India. The plant has a variety of traditional uses. Leaves are reported to possess demulcent, refrigerant properties, and are useful in cases of gonorrhoea, gleets and scalding urine. They are bruised in water and the filtrate is administered. Root is used as a tonic and diaphoretic and is given in mild cases of debility and fever. A decoction of it is said to be given as a demulcent in irritability of bladder and in gonorrhoea [1-4]. Hence, we

attempt to evaluate the leaves of *Sida spinosa* for anti diabetic potential in high fat diet and low dose streptozotocin induced diabetic rats and *in vivo* anti oxidant potential

MATERIALS AND METHODS

Plant materials: The leaves of *Sida spinosa*, were collected from southern part of India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India, Palayamkottai. The whole plant of *Sida spinosa* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered plant materials were stored in an airtight container.

Preparation of Extracts: The whole plant of *Sida spinosa* were dried in shade and powdered. The powdered plant materials were successively extracted with methanol by hot continuous percolation method in Soxhlet apparatus [5] for 24 hrs. The solvent from the extracts was recovered under reduced pressure using rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Experimental animals: Healthy adult male Wistar (150-200gm) were used in the present study. The animals were caged in clean polypropylene cages under controlled temperature 20-24°C and 12 h light / dark and were fed with standard rat pellet diet (Hindustan Lever Limited, Mumbai, India) and clean drinking water was made available ad libitum. All animal procedures were performed in accordance with the recommendations for the proper care and use of laboratory animals.

Animal diet

Control diet: Wheat flour 22.5%, roasted bengal gram powder 60%, skimmed milk powder 5%, casein 4%, refined oil 4%, salt mixture with starch 4% and vitamin & choline mixture 0.5%.

High fat diet: Wheat flour 20.5%, roasted bengal gram 52.6%, skimmed milk powder 5%, casein 4%, refined oil 4%, coconut oil 9%, salt mixture with starch 4% and vitamin & choline mixture 0.5%, cholesterol 0.4%.Induction of diabetes mellitus

Experimental induction of diabetes: Animals were allowed to fast for 12 h and after estimating the baseline blood glucose levels they were administered freshly prepared streptozotocin (STZ), 60 mg/kg b.w. i.p) in 0.1 mol/L cold citrate buffer, pH 4.5. The STZ treated animals were allowed to drink 5% glucose solution over night to overcome drug induced hypoglycemia. After 5 days of development of diabetes, rats with moderate diabetes having persistence glycosuria and hyperglycemia (blood glucose>250 mg/dL were considered diabetic and were used for further experimentation [6].

Experimental Design: Animals were divided into five groups, consisting of a minimum of six animals each:

- Group I: Non Diabetic Control (10 ml/kg normal saline)
- Group II: Diabetic control (STZ)
- Group III: STZ+ Methanolic extract of *Sida spinosa* (200 mg/kg, b.wt)
- Group IV: STZ+ Methanolic extract of *Sida spinosa* (400 mg/kg, b.wt)
- Group V: STZ+ Glibenclamide, 5 mg/kg.

All the drugs were administered orally and treatment was continued for 14 days. The fasting blood samples were collected on days 0, 5, 10 and15 to determine the blood glucose level. On day 15th blood samples were collected to estimate biochemical parameters. The doses employed for all drugs were within therapeutic range to suit the experimental animal used.

Determination of blood glucose level

Blood glucose concentration of overnight fasted animals was determined using standard kit (Ranbaxy, Glucose estimation Kit), based on the glucose oxidase method. Blood samples were withdrawn through the retro-orbital plexus of anaesthetized rats, using heparinised haematocrits.

Biochemical Estimation: At end of the experiment the blood samples were collected from the experimental animals for biochemical estimation and the animals were sacrificed by cervical dislocation method. Serum was analyzed for total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and Low density lipoprotein (LDL) levels using standard commercial diagnostic kits (Agappe Diagnostics, Kerala) following the manufacturers instruction in a semi auto analyzer (Mispa Excel Chemistry Analyser, Mumbai) and all the values were tabulated. Rats were sacrificed by cervical dislocation

under mild ether anesthesia on day 61 and tissues such as liver was removed and used for preparation of homogenate [7].

Statistical analysis: Values are expressed as mean ± standard error mean (SEM) and analyzed using statistical package for social sciences (SPSS) version 10 using ANOVA followed by Dunnett's test. P<0.05 were considered significant.

RESULTS AND DISCUSSION: Diabetes mellitus is possibly the world's largest growing metabolic disease and as the knowledge on the heterogencity of this disorder is advanced, the need for more appropriate therapy increases. Alternate strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries [8]. STZ is commonly used in chemically induced diabetic animal model. The timing of STZ injection is important and will affect the type of diabetes that subsequently develops. If STZ is injected to adult rats (i.e. 3 months or older) type 1 diabetes results. However, if injected during the first week of birth, the capacity of pancreatic b-cell re generation remains in the animals, type 2 diabetes develops [9].Table 1 shows the level of blood glucose in normal and experimental animals on 0, 5, 10 and 15 days of drug treatment. There was a significant reduction of blood glucose in methanolic extracts of *Sida spinosa* at the dose of 200 mg/kg and glibenclamide treated hyperglycemic animals compared to diabetic control animals. The hypoglyamic activity of methnolic extracts of *Sida spinosa* was compared with glibenclamide, a standard second generation hypoglycemic drug. Acute administration of sulfonyl urea increases insulin release from the pancreas. Sulfonyl ureas such as glibenclamide have been used for many years to treat diabetes, to stimulate insulin secretion from b-cells principally by inhibiting ATP-sensitive K+ (KATP) channels in the plasma membrane. Further, it is known that sulfonylureas have a direct effect on β-cell exocytosis and that effect is mediated by a mechanism that does not involve direct activation of protein kinase-C, which place a major role in controlling the b-cell potential [10]. The inhibition of ATP sensitive channels leads to membrane depolarization, activating Ca^{2+} channels, increased calcium influx, a rise in cytosolic (Ca^{2+}) and there by insulin release. Oral administration of methanolic extract of *Sida spinosa* and glibenclamide to the STZ-induced diabetic rats decreased the blood glucose levels.

Table 1: Effect of methanolic extract of *Sida spinosa* on glucose level in STZ induced diabetic rats (Values are

Groups	Serum glucose levels (mg/dl)			
	0 day	5th day	10th day	15th day
Vehicle control	104.56 ± 1.68	107.41 ± 2.32	103.75 ± 1.83	102.47 ± 1.84
STZ induced diabetic control	327.97 ± 2.46 a,#	338.80 ± 2.78 a,#	333.08 ± 1.46 a,#	328.89 ± 2.55 a,#
STZ + MESS (200mg/kg)	338.04 ± 10.83	317.93 ± 11.65 b,*	235.82 ± 9.81b,**	151.54 ± 6.83 b,**
STZ + MESS (400mg/kg)	335.72 ± 18.46	211.58 ± 7.66 b,**	138.93 ± 8.45 b,**	101.79 ± 1.49 b,**
STZ+Glibenclamide (5 mg/kg)	333.51 ± 4.38	230.47 ± 2.59 b,**	155.49 ± 2.83 b,**	116.58 ± 2.77 b,**

STZ (50mg/kg. b.w) was injected to control and all other drug treated groups; [a] STZ induced diabetic group *vs* normal group, #*p* <0.001; [b] extract treated group *vs* STZ induced diabetic group, [*] *p* <0.05; [**] *p* <0.001; MESS: Methanolic extract of *Sida spinosa.*

Table 2 shows the level of total cholesterol, triglyceride, LDL and HDL. Serum total cholesterol, triglyceride, LDL and HDL levels were significantly elevated in diabetic group when compared with control group animals. Increase in concentration of total cholesterol, triglycarides, LDL, VLDL and decreased HDL is observed in HFD+ low dose STZ untreated diabetic rats. Hyperlipidemia is a recognized consequence of diabetes mellitus. Administration of methanolic extracts of *Sida*

spinosa (200 & 400 mg/kg) and standard drug glibenclamide resulted in a significant fall of these serum lipoproteins when compared to diabetic rats. HDL level was decreased in the diabetic group when compared to the non diabetic control rats. A significant improvement in serum HDL was found in both extracts and standard drug treated animals. The group receiving methanolic extract of *Sida spinosa* (400mg/kg) had showed significant results than that of other groups.

Table 2: Effect of methanolic extract of *Sida spinosa* on serum lipid profiles in STZ induced diabetic rats after 14 days treatment (Values are mean±SEM, n=6)

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Vehicle control	148.05 ± 2.78	110.75 ± 2.95	122.43 ± 2.71	50.38 ± 1.45
STZ induced diabetic control	267.46 ± 2.90 [a,]	224.67 ± 1.83 [a, #]	226.26 ± 1.45 [a, #]	30.28 ± 1.15 [a, #]
STZ + MESS (200mg/kg)	198.49 ±4.81 [b,*]	179.01 ±2.84 [b,*]	172.53 ±3.78 [b,*]	34.62 ±2.47 [b,*]
STZ + MESS(400mg/kg)	163.69 ±4.52 [b,*]	145.83 ±3.59 [b,*]	143.58 ±1.38 [b,*]	40.75 ±0.93 [b,*]
STZ+Glibenclamide (5 mg/kg)	160.64 ±4.77 [b,*]	137.44 ±4.59 [b,*]	130.85 ±2.89 [b,*]	43.51 ±1.25 [b,*]

STZ (50mg/kg. b.w) was injected to control and all other drug treated groups; [a]STZ induced diabetic group *vs* normal group, [#]$p<0.001$; [b] treated group *vs* STZ induced diabetic group, [*]$p<0.001$. MESS: Methanolic extract of *Sida spinosa*

CONCLUSION: The methanolic extract of whole plants of *Sida spinosa* had showed significant antidiabetic and antioxidant activities in STZ induced diabetic rats. Furthermore, studies are required to isolate the active components present in the whole plants of *Sida spinosa* and explore their possible mechanism of action on antidiabetic activity.

REFERENCES:

1. Kirtikar KR., Basu BD. 1999 Indian Medicinal plant, I, International Book Distributors, Dehradun, Lass, p. 306 - 308.
2. The Wealth of India, 9, 1999, National Institute of Science Communication, P. 325.
3. Khar, Indian Medicinal Plant an illustrates Dictionary.
4. Charles B., Heiser, 2003, Needs in my garden, Timber Press, U.S.N. P.165
5. Harborne. Phytochemical methods 11 Edn.In Chapman &, Hall.New York, 1984; 4-5.
6. Pavana, Anti hyperglycemic and antilipidperoxidative effects of *Tephrosia purpurea* seed extract in streptozotocin induced diabetic rats. Indian Journal of Clinical Biochemistry, 2007; 22: 77-83.
7. Torrico, Hypoglycemic effect of *Croton cuneatus* in streptozotocin-induced diabetic rats. Brazilian Journal of Pharmacognosy, 2007; **17**:166-169.
8. WHO launches the first global strategy on traditional medicine: press release, 2002;38.
9. Szkudelski. The mechanism of alloxan and streptozotocin action in b-cells of the rat pancreas. *Physiological Resi*, 2001; 50: 536-546.
10. Davis (2001) Insulin, oral hypoglycemic agents and the pharmacology of the endocrine pancreas. In: Hardman, J.G., and Limbird., L.E., (eds) Goodman and Gilman's. The pharmacological basis of therapeutics 10th ed. New York: McGraw Hill Medical Publishing Division. 2001; 679-1710.