Evaluation of Anti-diabetic Activity of Ethanolic Extract of *Alternanthera sessilis* Linn. in Streptozotocin-induced Diabetic rats.

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ABSTRACT

The antidiabetic activity of ethanolic extract of whole plant of *Alternanthera sessilis* Linn. (EEAS) was investigated in a model of streptozotocin induced diabetes in rats. The biochemical parameters studied were: plasma glucose, degree of glycosylation of hemoglobin and peripheral consumption of glucose levels on 1, 3, 7 and 10th day. Ethanolic extract had shown significant protection and lowered the blood glucose levels to normal in glucose tolerance test. In streptozotocin induced diabetic rats the maximum reduction in blood glucose was observed after 2h at a dose level of 200 and 400 mg/kg of body weight. The streptozotocin induced diabetic rats showed significant reductions in biochemical parameter after treatment with the extract and Glibenclamide (used as standard) as compared to the diabetic controls. The ethanolic extract of *Alternanthera sessilis* Linn. exhibited antidiabetic activity and its sensitivity in experimentally induced diabetic rats in dose dependent manner. The current results clearly indicated the beneficial effects of the ethanolic extract of *Alternanthera sessilis* Linn. in both controlling hyperglycemia and the protection of the pancreatic islet cells against oxidative stress in the diabetic animals.

Keywords: Diabetes, Hypoglycemic effect, *Alternanthera sessilis* Linn., Streptozotocin, Ethanolic extract.

INTRODUCTION

Diabetes mellitus is a chronic and endocrine disorder caused by inherited and/or acquired efficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin production. Type II diabetes mellitus is a heterogeneous disorder characterized by a progressive decline in insulin action, followed by the pancreatic beta cell dysfunction [1]. Complications such as renal failure, coronary artery disorder, cerebrovascular disease, neurological complications, blindness, dyslipidemia, obesity, limb amputation and failure of various organs and eventually premature death are associated with chronic hyperglycemia [2]. It has been suggested that diabetes is the third leading cause death due to high level of morbidity and mortality in the developing countries. About more than 200 million people worldwide have diabetes mellitus and 300 million will acquire this disease by 2025 [3]. It has been estimated that Indian people are more genetically susceptible to diabetes accounting about 40 million and would reach up to 74 million by 2025 [4]. In ancient times, plants and herbs were used as remedy for serious health medicinal complications. Herbal drugs have lesser or no side effects and are less expensive as compared to synthetic drugs. Medicinal plants and their bioactive constituents are used for the treatment of diabetes throughout the world. Many indigenous Indian medicinal plants have been found to be useful for managing diabetes [5, 6]. After recommendation made by World Health Organization on medicinal plants for anti-diabetic drugs, many researchers focused on traditional medicinal plants for more effective and safer hypoglycemic agents. Many useful plants and herbs introduced in pharmacological and clinical trials have been confirmed their blood sugar lowering effect. So it is essential to know about the pharmacological evaluation of various plants used in the traditional system of medicine [7].

Alternanthera sessilis Linn. (Amaranthaceae) is an annual or perennial prostate herb with several spreading branches, bearing short petioled simple leaves and small white flowers, found throughout the hotter part of India, ascending to an altitude of 1200m [8]. The plant spreads by seeds, which are wind and water-dispersed and by rooting at stem nodes. Young shoots and leaves are eaten as a vegetable in Southeast Asia [9]. It is a weed of rice throughout tropical regions and of other cereal crops, sugarcane and bananas. Although it is a weed, it has many utilities. The leaves are used in eye diseases, cuts, wounds and antidote to snake bite; skin diseases [10]. It is also reported about the wound healing property of *Alternanthera sessilis* [11]. The plants aerial parts also have shown a hepatoprotective activity [12]. The degenerative and necrotic changes in the liver and kidney in Swiss mice, caused by oral administration of water extract of *Alternanthera sessilis* in high doses

through histopathological test were revealed [13]. Studies have been proved that the ethanolic extract of *Alternanthera sessilis* Linn. shows a significant antimicrobial activity against microorganisms like *Bacillus polymexia, Salmonella typhii, Candida albicans* etc [14].

Despite the presence of effective antidiabetic medicines in the pharmaceutical market, screening for bioactive substance from natural plants is still attractive because they contain substances that are effective and safe in diabetes mellitus. In the present study traditional medicinal plant has been selected for the hypoglycemic effect.

MATERIALS AND METHODS

Plant Material

The plant was identified by the botanists of the VR College, Nellore, Andhra Pradesh. After authentication, fresh aerial parts of the young and matured plants were collected in bulk from the rural belt of Jangalakandriga village, Nellore, Andhra Pradesh, India during early summer. After collection, plants were washed very carefully and clearly, dried under shade and then milled in to coarse powder by a mechanical grinder. The powder was kept in air tight container for further use.

Drugs and Chemicals

The drugs and chemicals are used for the study are of analytical grade.

Preparation of extract

The powdered plant material was defatted with petroleum ether and then extracted with ethanol (95%) in a soxhlet apparatus. The solvent was removed under reduced pressure, which obtained a greenish-black sticky residue, stored in a desiccators till further study.

Animals

Wistar albino rats of either sex, weighing 180-250 g were used for the study. The selected animals were housed in acrylic cages in standard environmental conditions (25-30^{0}C). They were allowed free access to standard dry pellet diet and water *ad libitum*. Swiss albino mice of either sex weighing between 25-30gm were acclimatized to laboratory condition for 10 days before commencement of experiment. They were also allowed free access to standard dry pellet diet and water *ad libitum*. These studies have been approved by Institutional animal ethical committee.

Acute toxicity Studies

The test was carried out as suggested by Seth *et. al*., 1972. Swiss albino mice of either sex weighing between 25-30g were divided into different groups of six animals each. The control group received normal saline (2 ml/kg, PO). The other groups received 100, 200, 300, 500, 600, 800 mg/kg of EEAS respectively through oral route. Immediately after dosing, the animals were observed continuously for the first 4 hours for any behavioral changes. They were then kept under observation up to 14 days after drug administration to find out the mortality if any [15].

Effect on oral glucose tolerance in rats

After overnight fasting, a 0-min blood sample was taken from the tip of the tail of each rat of different groups under mild ether anesthesia. Without delay a glucose solution (2g/kg) was administered by a gavage. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration. All blood samples were taken for the estimation of the blood glucose [16]. Estimation of blood glucose was carried out with the haemoglucostrips supplied by M/s Lifescan, Inc. USA with the help of a Johnson & Johnson ONE TOUCH blood glucometer.

Screening for antidiabetic activity (Induction to diabetes)

The acclimatized animals were kept fasting for 24 hours with water *ad libitum* and injected intraperitoneally a dose of 50 mg/kg of streptozotocin monohydrate in normal saline. After one hour, the animals were provided feed *ad libitum*. The blood glucose level was checked before and 72h after streptozotocin injection. The animals were considered diabetic when the blood glucose level was raised beyond 300mg/dl of blood. This condition was observed at the end of 72h after streptozotocin injection [17].

Single dose study

The animals were segregated into five groups of six rats in each. Group I rats were randomly selected from normal rats which received only distilled water. Group II to Group V animals were selected from the streptozotocin induced rats. Group II animals served as diabetic control. Group III and IV were treated with the extract 200 and 400 mg/kg respectively and group V animals received Glibenclamide (10 mg/kg). Blood samples were collected from the tip of tail of each rat under mild ether anesthesia at 0h, 1h, 2h and 4h after the administration of test samples [18-19]. Estimation of blood glucose was carried out with the haemoglucostrips supplied by M/s Lifescan, Inc. USA with the help of a Johnson & Johnson ONE TOUCH blood glucometer.

Multidose study

For multidose study, administration of test samples was continued for 10 days, once daily through oral route. Blood samples were collected from the tip of tail and the estimation of blood glucose was carried out as above on the 1, 3, 7 and 10^{th} day of the drug administration [18, 20].

Body weight

Body weights of all the animals were recorded just prior to and on the 10^{th} day of the study to determine the change in the body weight if any.

Determination of peripheral consumption of glucose *in-vitro*

Peripheral glucose consumption was studied in rat diaphragm preparation from animals fasted for 36 h prior to the experiment. The animals were sacrificed by cervical dislocation and the diaphragms were quickly taken out; followed by dividing each diaphragm into four pieces. The pieces of diaphragms were incubated in the nutrient solution with constant oxygenation and shaking (90 cycles / min) at 37^{o} C for 90 min in accordance with the procedure. The nutrient solution with the diaphragms was aerated for 10 min and used immediately. Glucose was added to a final concentration of 500 mg %. Each piece of diaphragm is incubated in 2.5 ml of glucose nutrient mixture. The results were expressed as glucose consumption per 10 mg of dry diaphragm (by subtracting glucose concentration after incubation from glucose concentration before incubation). The dry weight was determined after oven drying the diaphragm at 105^{o} C for 2 hours [21].

Statistical analysis

Statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnet's t-test. $P<0.05$ indicates significant difference between group means.

RESULT AND DISCUSSION

Acute toxicity

In acute toxicity study, it was found that the extract induced sedation and temporary postural defect at all tested doses. However, there was no mortality at any of the tested doses till the end of 14 days of observation.

Oral glucose tolerance test

Table 1 shows the blood glucose level of normal and experimental animals after oral administration of glucose (2g/kg). Extract treated and standard drug treated animals showed more significant decrease in peak blood glucose level after 1hr. After 2hrs the extract treated animals tend to bring the values near normal. It is very important to note that extracts showed a more pronounced action in glucose tolerance test.

Single dose study

The results of Table 2 reveals that the extract produced significant decrease in the blood glucose level when compared with the controls in streptozotocin induced hyperglycaemic rats in the single dose experiment at the tested dose level and are comparable with the standard drug Glibenclamide.

Multi dose study

In the multi dose study (Table 3), the test extract constantly maintained significant reduction of the glucose level in diabetic rats throughout the experimental period suggesting the anti-hyperglycaemic property of the extract. Diabetes mellitus causes failure to use of glucose for energy, which leads to increase utilization and decreased storage of protein responsible for reduction of body weight essentially by depletion of the body proteins.

Effect on body weight

The effect on the body weight also investigated properly. In Table 3 it was observed that the ethanolic extract reversed the weight loss of the diabetic rats and they returned to near normal.

Peripheral consumption of glucose

Streptozotocin causes irreversible destruction β-cells of pancreas. Thus the anti hyperglycemic activity might be due to extra pancreatic mechanism. Hence, the effect of ethanolic extract of *Alternanthera sessilis* aerial parts on peripheral consumption of glucose was investigated. The result suggests that the extract produces an antidiabetic action mediated by an increase in peripheral glucose consumption in the rat diaphragm of diabetic rats, especially at a concentration of 600µg/ml (Table-4). Insulin increased the peripheral glucose consumption in normal and diabetic rats. Thus, the extract might have insulin like activity and the anti-hyperglycaemic effect of the extract might be due to an increase in peripheral glucose consumption.

CONCLUSION

The ethanolic extract of *Alternanthera sessilis* aerial parts exhibited significant hypoglycemic activity in streptozotocin induced diabetic rats. From the phytochemical analysis it was found that the major chemical constituent of the extract was saponins [22]. On the basis of above evidence it is possible that the presence of saponins may be responsible for the observed antidiabetic activity. Thus, the saponins in the extract may be suspected to possess the activity that may be attributed to their protective action on lipid peroxidation and at the same time the enhancing effects on cellular antioxidant defense contributing to the protection against oxidative damage in streptozotocin induced diabetes [23-24]. Further pharmacological and biochemical investigations are underway to find out the active constituents responsible for antidiabetic activity and to elucidate its mechanism of action.

RESULTS

Table 1: Effect of ethanolic extract of *Alternanthera sessilis* Linn. aerial parts (200 and 400mg/ kg, PO), on oral glucose tolerance test (OGTT) in normal and streptozotocin induced diabetic rats.

Groups	Treatment	Blood sugar level (mg/dl)				
		Fasting	30 min	60 min	90 min	120 min
I	Normal	75.00 ± 0.77	149.83 ± 2.32	176.83 ± 2.09	125.17 ± 2.83	80.16 ± 1.83
II	Diabetic control (Streptozotocin only)	250.33 ± 3.10	322.33 ± 4.16	374.17 ± 5.16	319.33 ± 3.29	317.83 ± 2.67
III	Diabetic + Extract (200mg/kg)	72.69 ± 1.79	147.45 ± 0.95	169.23 ± 3.03*	135.47 ± 3.82*	88.75 ± 4.01
IV	Diabetic + Extract (400mg/kg)	69.54 ± 1.62	138.87 ± 3.01	166.56 ± 3.69*	123.21 ± 2.95*	85.48 ± 1.54
V	Diabetic + Glibenclamide (10mg/kg)	76.50 ± 2.02	151.56 ± 3.45	185.33 ± 2.53*	126.83 ± 2.46*	92.50 ± 1.50

Values are given as Mean ± SEM for n=6

Group II was compared with group I. Group III, IV and V were compared with group II.

Values are statistically significant at *p< 0.05.

Table 2: Effect of single dose treatment of ethanolic extract of *Alternanthera sessilis* Linn. aerial parts on blood glucose level in normal and Streptozotocin induced diabetic rats.

Group	Treatment	Blood glucose level(mg/ dl)			
		Basal value	1hr	2hr	4hr
I	Normal	76.33 ± 0.71	76.17 ± 0.65	75.83 ± 0.95	76.17 ± 0.79
II	Diabetic control (Streptozotocin only)	349.67 ± 2.95	350.17 ± 2.71	349.83 ± 2.62	350.17 ± 2.79
III	Diabetic + Extract (200mg/kg)	335.50 ± 3.02NS	285.33 ± 4.75*	280.83 ± 3.21*	269.83 ± 2.98*
IV	Diabetic + Extract (400mg/kg)	325.72 ± 2.45NS	279.33 ± 1.46*	271.63 ± 2.45*	260.45 ± 2.75*
V	Diabetic + Glibenclamide	343.17 ± 5.12NS	319.50 ± 5.35*	298.83 ± 3.91*	284.83 ± 3.65*

Values are mean ± SEM for n=6

*P < 0.05 = significant; NS = Not significant.

Group II was compared with group I while Group III, IV and V were compared with group II.

Table 3: Effect of multiple dose treatment of ethanolic extract of *Alternanthera sessilis* Linn. aerial parts (Once daily), on blood glucose level and change in body weight after 10 days in normal and Streptozotocin induced diabetic rats.

Group	Treatment	Blood glucose level (mg/ dl)					Change in body weight (g)
		Basal value	Day 1	Day 3	Day7	Day 10	
I	Normal	76.33 ± 0.71	76.17 ± 0.48	75.83 ± 0.40	76.17 ± 0.65	76.50 ± 0.56	(+) 9.83 ± 1.47
II	Diabetic control (STZ only)	349.67 ± 2.95	356.83 ± 2.83	353.83 ± 3.39	354.33 ± 3.90	354.17 ± 3.83	(-) 8.83 ± 0.87
III	Diabetic + Extract (200mg/kg)	338.77 ± 3.19^{NS}	230.56 ± 2.79*	219.24 ± 3.65*	210.02 ± 2.23*	202.01 ± 2.01*	(+) 9.01 ± 1.01*
IV	Diabetic + Extract (400mg/kg)	329.58 ± 3.02^{NS}	211.56 ± 3.45*	211.23 ± 3.78*	198.54 ± 2.54*	179.92 ± 1.91*	(+) 8.78 ± 1.25*
V	Diabetic + Glibenclamide	343.17 ± 5.12^{NS}	264.33 ± 4.07*	235.83 ± 3.57*	219.33 ± 4.28*	205.33 ± 3.65*	(+) 8.83 ± 0.98*

Values are mean ± SEM for n=6; $^{*}P < 0.05$ = significant; NS = Not significant.

Group II was compared with Group I while Group III, IV and V were compared with Group II.

Table 4: Effect of ethanolic extract of *Alternanthera sessilis* Linn. on *in-vitro* peripheral glucose consumption in diaphragm of normal and diabetic rats.

Group	Glucose consumption (mg/10mg of diaphragm dry weight)			
	Control	Ethanolic extract (µg/ ml)		Insulin
		300 (µg/ ml)	600 (µg/ ml)	5 U/ ml
Normal rats	0.47 ± 0.03	0.59 ± 0.05^{NS}	0.68 ± 0.05*	0.82 ± 0.06*
Diabetic rats	0.49 ± 0.03	0.62 ± 0.11*	0.75 ± 0.07*	0.91 ± 0.07*

Values were expressed as Mean ± SEM for n=6.

$^{*}P < 0.05$ = Significant; NS = Not significant compared to control

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