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Anti-diabetic Activity of *Alternanthera Sessilis* Linn. Leaf Extract on Alloxan Induced Diabetic Rats

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ABSTRACT: *The present study was designed to investigate the anti-diabetic (anti-hyperglycemic) effect of 50% ethanolic extract of* Alternanthera sessilis *(Amaranthaceae) leaves on normal and alloxan induced diabetic rats. Diabetes was induced into male albino wistar rats by intraperitonial administration of alloxan at a does of 120 mg/kg body weight. The* Alternanthera sessilis *leaf extract was administered orally at three different doses to diabetic rats for 28 days. The diabetic rats showed an increase in levels of blood glucose and decrease in the levels of liver glycogen. Treatment with 50% ethanolic extract of* Alternanthera sessilis *leaves significantly reduced the levels of blood glucose and increased the levels of liver glycogen. Thus, the data suggests that* Alternanthera sessilis *leaf extract possesses an anti-hyperglycemic activity. The 450 mg/kg body weight dose of the extract is more effective than 150 mg/kg or 300 mg/kg doses of the leaf extract and to Glibenclamide (600 µg / kg body weight), the widely used allopathic drug.*

Keywords: Alternanthera sessilis *– Anti-hyperglycaemia –Alloxan.*

1. INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with the ling term damage dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (ADA 2007).

India, china and United States are supposed to be the countries having the largest number of diabetic patients in the year 2025. The WHO reported that 366 million people would suffer from Diabetes mellitus by the year 2030 (Wild *et al*. 2004).

Medicinal plants are used for their bioactive constituent for the treatment of Diabetes mellitus throughout the world especially in countries where access to the conventional modern therapies with anti-DM agents are inadequate and poverty is more among the rural populations (Ganesan *et al* 2007). Although several medicinal plants have gained importance for the treatment of DM many remain to be scientifically investigated (Punitha 2006).

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The Medicinal plant *Alternanthera sessilis* a very popular and fast spreading water weed that belongs to the Amaranthacae family was found to possess anti - diabetic properties in the ancient literature and used in folklore medicine. The present study was conducted to scientifically investigate the anti - diabetic activity of *Alternanthera sessilis* in alloxan induced diabetic rats.

2. MATERIAS AND METHODS

2.1. Animals

Male Albino rats of Wistar strain (130 ±20 gm) were used for the study procured from the laboratory animal house, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu. The animals were maintained under standard laboratory conditions of controlled temperature and humidity (at a room temperature of 22 ^{o}C - 24 ^{o}C with 12 hour day and night cycle) and were allowed to get acclimatized to standard laboratory diet supplied by M/s Hindustan Lever Ltd. Bangalore, India and fed with filtered water *ad libitum*. Ethical clearance for the handling of experimental animal was obtained from the Institutional Animal Ethics Committee (IAEC) constituted for the purpose and

the care of laboratory animals and taken as per the guidance of the Committee for the Purpose of Control and Supervision on Experimental in Animals (CPCSEA), ministry of Social Justice and Empowerment, Government of India (CPCSEA No: 158/1999/CPCSEA).

2.2. Experimental Induction of Diabetes

Diabetes mellitus was induced in the albino rats by administering alloxan monohydrate . animals were allowed to fast for 13 hours and were injected with freshly prepared alloxan monohydrate (120 mg/kg body weight, i.p.,) in sterile normal saline (Vijayavirgia *et al.* 2000). The animals were maintained in the diabetic state over a period of 28 days. Serum glucose was measured by reported method (Trinder P. 1969). After 72 hours of alloxan injection, the diabetic rats with glucose level > 250mg/dl were selected for the study (Perfumi *et al*. 1996).

2.3. Plant Material

Alternanthera sessilis was collected from plants grown in Tamilnadu Agricultural University (TNAU)Campus, Coimbatore, Tamil Nadu, identified and confirmed by the Taxonomists, Botanical Survey of India, Coimbatore , Tamil Nadu, India.

2.4. Preparation of Plant Extract

The leaves were shade dried, the powdered form of leaves of *Alternanthera sessilis* was taken and subjected to successive solvent extraction. The extraction was carried out with 50% ethanol . The solvent was distilled , evaporated and vacuum dried as per the standardized procedure (Rupashree *et al* 1999).

2.5. Phytochemical Analysis

The various extracts of leaves of *Alternanthera sessilis* were subjected to the various tests for identification of its active constituents by standard methods. Carbohydrates were identified by Molisch test, Proteins were identified by Biuret test, Triterpenoids and Steroids were identified by Libbermann-Burchard test, Alkaloids were identified by Dragendroffs method, Tannins were identified by Braemers test, Glycosides were identified by Legals test, Saponins were identified by Haemolysis test , Flavonoids were identified by Lead Acetate test, and Fixed oils were identified by the presence of oil stains on the filter paper (Nandhakumar Jothivel *et al* 2007).

2.6. Experimental Set Up

The experimental rats were divided into six groups of six animals in each group.

Group I : Animals served as normal healthy controls

GroupII : Untreated diabetic control rats

Group III : Diabetic rats were given leaf extract of *Alternanthera sessilis* (150 mg/kg, p.o) at a single dose per day.

Group IV : Diabetic rats were given leaf extractr of *Alternanthera sessilis* (300mg/kg, p.o) at a single dose per day

Group V : Diabetic rats were given leaf extract of *Alternanthera sessilis* (450mg/kg, p.o) at a single dose per day

GroupVI : Diabetic rats were given Glibenclamide (650 µg/ kg body weight) at a dose per day orally.

Body weights of the animals were recorded every week.

2.7. Collection of Blood, liver, Kidney, Pancreas from the Rat

After the experimental regimen, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected from cardiac puncture and serum was separated by centrifugation at 2500 rpm. The liver, kidney, pancreas was excised immediately and washed thoroughly in ice cold saline. The serum and tissue were collected and used for Biochemical estimation.

2.8. Estimation of Biochemical Parameters

Serum Glucose was estimated by GOD/POD method (Trinder 1969). Liver Glycogen was estimated by the method of Wieland 1963.

2.9. Statistical Evaluation

All results are expressed as Mean ±SD. Statistical Evaluation was done using One Way Analysis of Variance(ANOVA), followed by Student- t test

3. RESULT

3.1. Phytochemical Analysis

Compounds of different polarity from dried leaves of *Alternanthera sessilis* was extracted by sequential extraction process using different solvents such as Petroleum Ether, Chloroform, Acetone, Ethanol and Water. These sequential extracts were subjected due to presence of different chemical groups. Of all extract tested Ethanol extract was found to contain the highest numbers of phytochemicals such as Carbohydrates , Proteins, Alkaloids, Flavonoids, Saponin.

3.2. Body Weight

Body Weight increased significantly in all groups except Group II. All animals ingested normal amount of food and water during the study period.

3.3. Biochemical Parameters

Body weight, Serum glucose and liver glycogen levels in rats of different groups are shown in Table 1, 2 and 3. The glucose level was significantly high in Group II compared with Group 1. On the other hand, the level of serum glucose was significantly decreased in Group III, Group IV and Group V and Group VI when compared with Group II. It is evident from Table 2 that untreated diabetic rats when orally administered with 50% Ethanolic leaf entract of *Alternanthera sessilis* elicited a significant anti-diabetic activity. The decrease in blood glucose level of diabetic rats treated with extract might be due to elevated secretion of insulin, which in turn may increase the utilization of glucose at that time and may prevent weight loss .

Likewise from table 3 the glycogen content of the liver was significantly decreased when compared with the control group in diabetic rats, and the level restored maximum to the normal with the increase in the concentration of drug. The prevention of depletion of glycogen in liver tissue was possibly due to stimulation of insulin release from β cells that activate the Glycogen Synthase system. (Kamala Kannan 2003, Kamalakannnan 2005).

Table 1
Effect of 50% Ethanolic Extract of *Alternanthera sessilis* On Body Weight of the animal (in gms)

Groups	*0 days*	*7th day*	*14th day*	*21st day*	*28th day*
GroupI	130.16 ±7.67	133.00±7.79	134.50±7.96	136.50±7.96	142.00±8.78
GroupII	144.66±8.73	144.50±8.28	141.66±8.80	137.00±7.72	133.50±7.00
GroupIII	149.50±9.69	154.00±8.78	155.66±8.64	157.00±9.48	159.83±9.53
GroupIV	148.00±8.60	152.50±8.96	154.33±8.84	156.16±8.75	157.66±8.73
GroupV	151.16±8.75	155.00±8.67	156.00±8.60	167.66±4.36	163.00±10.43
GroupVI	153.50±10.5	156.50±9.69	167.83±4.44	163.00±10.43	164.66±10.48

Values are represented as mean ± SD of six animals.

Table 2
Effect of 50% Ethanolic Extract of *Alternanthera sessilis* on Blood Glucose (in mg/dl)

Group	*0th day*	*7th day*	*14th day*	*21st day*	*28th day*
GroupI	116.42±5.52	118.09±5.93	119.38±6.00	119.78±5.63	121.52±6.23
GroupII	270.42±18.5*	271.41±20.27*	275.82±20.32*	284.42±21.42*	283.71±21.72*
GroupIII	263.40±20.23	249.31±17.54	234.70±17.4	220.83±14.83	195.86±16.65
GroupIV	266.52±19.26	239.75±17.44	227.89±15.76	169.35±11.88	122.80±18.31
GroupV	274.44±21.60	230.13±15.73	181.65±12.58	142.76±8.90	124.40±6.92
GroupVI	267.18±21.7	250.52±18.38	209.85±14.87	160.53±10.14	124.51±6.93

Values are represented as mean ± SD of six animals. * Significantly different from normal rats ($p<0.05$)

Table 3
Effect of 50% Ethanolic Extract of *Alternanthera sessilis* on Liver Glycogen(mg/gm)

Group I	*Group II*	*Group III*	*Group IV*	*Group V*	*Group VI*
53.05±3.45	27.94±1.92	35.95±2.39	49.16±3.31	48.55±3.35	37.14±2.38

Values are represented as mean ± SD of six animals.

Table 4
Effect of 50% Ethanolic Extract of Alternanthera Sessilis on Protein,Urea,Creatinine & Hexokinase

	Control	*Diabetic control*	*Treatment*
Protein (mg/gm)	186.85±35.11	179.20±10.53	200.09±11.9
Urea (mg/dl)	33.40±2.05	48.46±3.12	36.96±2.30
Creatinine (mg/dl)	1.12±4.8	3.12±0.24	1.93±0.12
Hexokinase (units /hour/mg of protein)	0.270±2.10	0.13±7.96	0.26±2.01

Values are represented as mean ± SD of six animals.

In the present study, hexokinase activity was decreased significantly in the liver of diabetic rats as reported earlier (Grover *et al* 2000). Treatment with *Alternanthera sessilis* and glibenclamide may increase insulin secretion which in turn activates hexokinase thereby enhancing glucose utilization resulting in total decrease in blood sugar level.

CONCLUSION

Alloxan, a β – cytotoxin induces chemical diabetes in a wide variety of animal species by damaging the insulin – secreting β cells of the pancreas. Alloxan causes time and concentration dependent degenerative lesions of the pancreatic β cells (Lenzen 1988), Oberley 1988). Like that of Streptozotocin, Alloxan induced diabetes in rats is characterized by severe loss in body weight (Chen and Lanvzzo 1982) and due to loss or degradation of structural protein which are known to contribute to body weight. In the present study, a significant weight loss was observed in the diabetic group and significant improvement in weight was observed in groups treated with 50% Ethanolic extract of *Alternanthera sessilis* leaves.

The fundamental mechanism underlying hyperglycemia in DM involves over production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by tissues (Latner 1958). In the present study, we observed that oral administration of *Alternanthera sessilis* results in a significant reduction in blood glucose and 450 mg/kg body weight dose exhibited maximum reduction when compared to other two lower doses. To our best of knowledge this is the first reported scientific study and proof of anti-diabetic activity of the traditional medicinal plant *Alternanthera sessilis.*

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