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ANTI-DIABETIC POTENTIAL OF METHANOLIC EXTRACT OF *AERVA TOMENTOSA* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The purpose of the study is to investigate anti-diabetic potential of methanolic extract of *Aervatomentosa*in streptozotocininduced diabetes in rats. Animals were divided into different groups (n=6) and induced with STZ expect control group. After confirming the animals were diabetic, they were treated with methanol extracts (200 and 400 mg/kg) for a period of 60 days. At end of the experiment, the blood samples were collected and estimated biochemical parameters,Further, rats were sacrificed by cervical dislocation method for organ separation to check the toxicity in tissues. It was quite interesting to observe that there was marked reduction in blood glucose levels after treatment of methanol extracts of *Aerva tomentosa*(200 and 400 mg/kg). It also reduced theserum marker enzymes and lipid level towards normal control groups.This study provides scientific evidence that the methanol extract of *Aerva tomentosa*have anti-diabetic efficacy without showing any kind of toxicity upon tissues.

INTRODUCTION:

Diabetes mellitus is a complex disorder that characterized by hyperglycemia resulting from a malfunction in insulin secretion and/or insulin action both caused by impaired metabolism of glucose, lipids and protein [1, 2]. Diabetes mellitus is also associated with an increased risk for hypertriglyceridemia and hypertension [3]. Insulin therapy and oral hypoglycemic agents offer effective glycemic control; yet, their shortcomings limit their usage [4].

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The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs [5]. It is a common endocrine disorder in which there occur increased food and water intake[6] and characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both[7]. The world prevalence of diabetes among adults (aged 20–79 years) will be 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7% and 439 million adults by 2030. Between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries[8].

Aerva tomentosa(flowers) belongs to the family of Amaranthaceae. The plant has naturalised in northern regions of Australia and is cultivated

and utilised by the indigenous peoples. *Aerva tomentosa* is a deciduous shrub, widely distributed in Punjab, Rajasthan and Gujarat. It is diuretic, demulcent, purgative and emetic. Flowers and seeds are used against swelling, headache and rheumatism[9,10,11].As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity[12]. Roots and flowers are reported to possess medicinal properties against rheumatism and kidney problems. Plant is reported as anthelmintic, diuretic and demulcent[13]. The decoction of the plant is administered to remove swellings[14], applied to acne like conditions of the face[15]. There is a lack of scientific reports revealed that the antidiabetic activity of methanol extract of hole plant of *Aerva tomentosa.* Hence, The aim of the present study was to evaluate the antidiabetic and antioxidant potential of methanol extract of *Aerva tomentosa*in Streptozotocin induced diabetic rats

MATERIAL AND METHODS

Plant materials

The whole plant of *Aerva tomentosa* were collected from Tamil Nadu and authenticated by Dr. V. Chelladurai, Research Officer, Dept. of Botany, Central Council for Research in Ayurveda & Siddha, Govt. of India.The whole plant of *Aerva tomentosa*were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of extracts

The above powdered materials were successively extracted with methanol by hot continuous percolation method in Soxhlet apparatus[16]for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. The extracts were suspended in 2% tween 80[17].

Experimental Animals

Albino Wister rats of 16-19 weeks age, weighing 150-175g were procured from the Animal House, SreeVidyanikethan College of Pharmacy, Tirupati. The animals were kept in cages, 2 per cage, with 12:12 hr light and dark cycle at $25^0 \pm 2^0$C. The animals were maintained on their respective diets and water.Animal Ethical Committee's clearance was obtained for the study.

Induction of diabetes

Diabetes was induced in rats by the intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 55 mg/kg dissolved in distilled water (1 ml/kg b.w.). Seven days after the injection, the blood glucose levels were measured. Each animal with a blood glucose concentration level above 250 mg/dl was considered to be diabetic and used in the experiments. To prevent the hypoglycemia which occurred during the first 24 h following the STZ administration, 5% glucose solution was orally given to the diabetic rats. In all experiments, rats were fasted for 16 h prior to STZ injection[18].

Determination of hypoglycemic effect on diabetic rats

Diabetic animals were divided into five groups of six animals each. Group I served as control. Remaining groups received the methanol extract of *Aerva tomentosa* (200mg/kg & 400mg/kg body weight) and glibenclamidewas given as, once a day for 60 days. The fasting blood samples were collected on day 0, 7, 15, 30 day 45 and day 60 to determine the blood glucose level. On day 60 blood samples were collected to estimate biochemical parameters. Immediately after the collection of blood samples the rats were sacrificed by cervical dislocation and tissues were excised to measure their antioxidant andliver glycogen status.

Experimental design

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

Group I: Control rats (10 ml/kg normal saline)
Group II: Diabetic control (Streptozotocin at the dose of 55mg/kg)
Group III : STZ+ 200 mg/kg methanol extract of *Aerva tomentosa*
Group IV: STZ+ 400 mg/kg methanol extract of *Aerva tomentosa*
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 day 0, 7, 15, 30,45and day 60 to determine the glucose level. At the end of the experimental period, the blood samples were collected from the retro-orbital plsexus using microcapillary technique. After blood collection, all the rats were sacrificed by euthanasia. Organs such as the liver, and pancreas were excised immediately and washed with ice cold saline solution. The doses employed for all drugs were within the therapeutic range to suit the experimental animal used.

Estimation of blood glucose

Glucose was determined using the system Accu-Check Active glucometer (Roche Diagnostics India Pvt. Ltd, India).

Acute Oral Glucose Tolerance Test (OGTT) in normal and STZ-diabetic rats

The oral glucose tolerance test (OGTT) was performed for two different extracts doses (200 & 400mg/kg body weight) of methanol extract of *Aervatomentosa*&glibenclamide in normal and STZ induced diabetic rat model. Four days after diabetes induction, the OGTT was performed by feeding glucose in the form of a solution through orally to STZ induced diabetic rats fasted for 18 hours. One hour later, glucose (2 g/kg) was administrated. Blood glucose levels were determined from the tail vein at 0, 15, 30, 60, 90and 120min after glucose administration using one touch glucometer (Accu-check).

Biochemical estimation

Plasma samples were analyzed for total cholesterol, HDL-cholesterol and triglycerides were estimated using Boehringer Mannheim kits by Erba Smart Lab analyzer USA. LDL-cholesterol and VLDL-cholesterol were calculated by using Friedwald method[19].Ester cholesterol[20] and free cholesterol[20] were analyzed by using digitonin. Portions of liver, and pancrease tissues were blotted, weighed and homogenized with methanol (3 volumes) and the lipid extracts were obtained by the method of Folch*et al*[21]. Extracts were used for the estimation of ester cholesterol and free cholesterol, triglycerides[22], and phospholipids[23]. Plasma total cholesterol: HDL-cholesterol ratio and LDL-cholesterol: HDL-cholesterol ratio was also calculated.

STATISTICAL ANALYSIS

The results are expressed as mean ± SEM. Comparison between the treatment groups and control were performed by one-way analysis of variance (ANOVA) followed Tukeys multiple Comparison tests.

RESULTS AND DISCUSSION

Effect of *Aerva tomentosa* on glucose level in STZ induced diabetic rats

Table-3 shows the level of blood glucose in normal and experimental animals on 0, 7, 15, 30, 45 and 60 days of drug treatment. There was a significant reduction of blood glucose in various extracts of *Aerva tomentosa* at the dose of 400 mg/kg and glibenclamide treated hyperglycemic animals compared to diabetic control animals. The hypoglyamic activity of various extracts of *Aerva tomentosa* was compared with glibenclamide, a standard second generation hypoglycemic drug. Acute administration of sulfonylurea 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 (Davis and Granner, 2001). The inhibition of ATP sensitive channels leads to membrane depolarization, activating Ca2+ channels, increased calcium influx, a rise in cytosolic (Ca2+) and there by insulin release. Oral administration of various extracts of *Aervatomentosa* and glibenclamide to the STZ-induced diabetic rats decreased the blood glucose levels.

Effect of *Aerva tomentosa* serum biomarkers in STZ induced diabetic rats

Table-4 shows the body weight of control and experimental animals. Results showed no intra group variation in the basal body weight. There was significant reduction of body weight in diabetic control animals compared to test drug treated animals. The 0 day body weight showed no difference in all groups. A significant reduction of body weight was observed in diabetic control animals and drug treated animals when compared to control rats. Reduced body weight indicates the induction of diabetes. The decreased body weight in diabetic rats is due to excessive break down of tissue protein. The normal control and drug treated rats gained significant weight ($P<0.01$) at the end of the experiment. Treatment with various extracts of *Aervatomentosa* and glibenclamide improved body weight significantly inducing prevention of muscle wasting due to hyperglycemic condition (Ravi *et al*., 2004).

Effect of *Aerva tomentosa* on serum lipid profiles in STZ induced diabetic rats

Table-5 shows the level of serum lipoproteins such as 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 STZ untreated diabetic rats, Hyperlipidemia is a recognized consequence of diabetes mellitus. Administration of various extracts of *Aerva tomentosa* at the dose of 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. After 60 days of various extracts of *Aervatomentosa* and glibenclamide supplementation, there was a significant elevation in HDL level in serum and the results were found to be similar to that of control rats. The group receiving methanolic extract of *Aerva tomentosa* had showed significant results than that of other extract treatment groups.

Effect of *Aerva tomentosa* on liver glycogen level in STZ induced diabetic rats: Table-6 shows the significant reduction in liver glycogen found in Group II rats. During diabetes liver shows decrease in weight due to enhanced catabolic process such as glycogenolysis, lipolysis and proteolysis, which is the outcome of lack of insulin and or cellular glucose in liver cells. Administration of methanolic extract of *Aerva tomentosa* at the dose of 400mg/kg were shows the significant increment in liver glycogen content than that of low dose extract treated group animals.

Note:STZ (55mg/kg. b.w) was injected to all 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

	Serum glucose levels (mg/dl)					
Groups	**0 day**	**7th day**	**15th day**	**30th day**	**45th day**	**60th day**
Vehicle control	104.59 ± 0.25	105.78 ± 0.22	103.41 ± 0.08	105.68 ± 0.17	105.18 ± 0.20	108.18 ± 0.34
STZ induced diabetic control	296.01 ± 0.26 [a,#]	297.53 ± 0.10 [a,#]	298.93 ± 0.13 [a,#]	296.61 ± 0.07 [a,#]	298.77 ± 0.52 [a,#]	292.27 ± 0.14 [a,#]
STZ + *Aerva tomentosa* (200mg/kg)	295.28 ± 0.24	278.21 ± 0.26 [b,**]	271.87 ± 0.16 [b,*]	264.11 ± 0.21 [b,*]	259.61 ± 0.06 [b,*]	241.11 ± 0.24 [b,*]
STZ + *Aerva tomento-sa*(400mg/kg)	296.67 ± 0.08	268.47 ± 0.19 [b,**]	229.87 ± 0.17 [b,*]	208.54 ± 0.98 [b,**]	193.54 ± 0.48 [b,**]	168.87 ± 0.33 [b,**]
STZ+Glibenclamide (5 mg/kg)	297.63 ± 0.10	261.76 ± 0.18 [b,**]	221.52 ± 0.03 [b,**]	206.31 ± 0.24 [b,**]	179.47 ± 0.25 [b,**]	155.14 ± 0.20 [b,**]

Table 1: Effect of*Aervatomentosa*on glucose level in stz induced diabetic rats(Values are mean±SEM, n=6)

Groups	**SGOT (IU/dl)**	**SGPT (IU/dl)**	**ALP (IU/dl)**
Vehicle control	63.76 ± 0.24	68.50 ± 0.10	108.01 ± 0.18
STZ induced diabetic control	161.07±0.08 [a, #]	157.02 ± 0.22 [a, #]	196.15 ± 0.21 [a, #]
STZ + *Aerva tomentosa* (200mg/kg)	94.63 ± 0.102 [b,*]	96.45 ± 0.07 [b,*]	154.16 ± 0.19 [b,*]
STZ + *Aerva tomento-sa*(400mg/kg)	82.05 ± 0.17 [b,*]	86.79 ± 0.23 [b,*]	129.52 ± 0.08 [b,*]
STZ+Glibenclamide (5 mg/kg)	68.82 ± 0.23 [b,*]	77.50 ± 0.11 [b,*]	122.17 ± 0.16 [b,*]

Table 2: Effect of *Aervatomentosa* serum biomarkers in stz induced diabetic rats (Values are mean±SEM, n=6)

Note:STZ (55mg/kg. b.w) was injected to all 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.

Table 4: Effect of *Aervatomentosa* on liver glycogen level in stz induced diabetic rats (Values are in mean±SEM, n=6)

Groups	**Liver glycogen (mg/gm of liver)**
Vehicle control	44.39±0.30
STZ induced diabetic control	12.89±0.16 [a,#]
STZ + *Aerva tomentosa* (200mg/kg)	28.83±0.19 [b,*]
STZ + *Aerva tomentosa* (400mg/kg)	34.05±0.25 [b,*]
STZ+Glibenclamide (5 mg/kg)	35.38±0.09 [b,*]

Note:STZ at the dose of 55mg/kg.b.w was injected to all drug treated groups; [a] STZ induced diabetic group *vs* normal group, [#]*p*< 0.001; [b] treated group *vs* STZ induced group, [*]*p*< 0.001.

Table 3: Effect of A*ervatomentosa* on serum lipid profiles in STZ induced diabetic rats(Values are mean±SEM, n=6)

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Vehicle control	150.09 ± 0.17	111.23 ± 0.20	123.59 ± 0.08	50.55 ± 0.05
STZ induced diabetic control	270.65 ± 0.20 [a, #]	225.43 ± 0.24 [a, #]	226.22 ± 0.25 [a, #]	30.59 ± 0.09 [a, #]
STZ + *Aerva tomentosa* (200mg/kg)	198.62 ±0.19 [b,*]	179.04 ±0.26 [b,*]	178.51 ±0.07 [b,*]	35.41 ±0.07 [b,*]
STZ + *Aerva tomentosa* (400mg/kg)	164.08 ±0.24 [b,*]	145.51 ±0.18 [b,*]	145.53 ±0.12 [b,*]	41.86 ±0.16 [b,*]
STZ+Glibenclamide (5 mg/kg)	161.01 ±0.25 [b,*]	139.56 ±0.03 [b,*]	136.40 ±0.19 [b,*]	45.42 ±0.21 [b,*]

Note:STZ (55mg/kg. b.w) was injected to all drug treated groups; [a]STZ induced diabetic group *vs* normal group, [#]$p<0.001$; [b] treated group *vs* STZ induced diabetic group, [*]$p<0.001$.

CONCLUSION

This study provides scientific evidence that the methanol extract of *Aerva tomentosa* have anti-diabetic activity. Therefore, further investigations are required to gain more insight in to the possible mechanism of action.

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