

Research Article

Antidiabetic and Antihyperlipidemic Activity of Ethanolic Extract of the leaf of *Asperagus racemosus* on Streptozotocin Induced Diabetes Rats

N. Lal Mahammed^{1*}, G. Jyothi¹, T. Narendra Chary², CH. Venkateswara Reddy¹
and G. Nagarjuna Reddy¹

¹KLR Pharmacy college, Paloncha, Khammam (Dist), Andhra Pradesh, India.

²Department of Pharmacy, MITS College of Pharmacy, Madhira Nagar, Chilkur (V&M), Kodad-508206, Nalgonda dist, Andhra Pradesh, India.

ABSTRACT

The objective of the present work is to study the antidiabetic and antihyperlipidemic effect of ethanolic leaf extract of *Asperagus racemosus* in streptozotocin (STZ) induced diabetes in rats. The diabetes was induced by single dose of STZ (55 mg/kg) in citrate buffer, while the normal control group was given the vehicle (Normal saline) only. After three days of induction of diabetes, the diabetic animals were treated further four weeks with ethanolic leaf extract of *Asperagus racemosus* (200 mg/kg and 400 mg/kg) and glipizide (2.5 mg/kg). Blood glucose estimation was performed every week of the study. At the end of study period, animals were sacrificed for biochemical studies. STZ-induced diabetic rats showed marked hyperglycemia, hypertriglyceridemia and hypercholesterolemia at the end of study period. Body weight is significantly increased in diabetic rats. The four week treatment with ethanolic leaf extract of *Asperagus racemosus* (200 mg/kg and 400 mg/kg) significantly ameliorated the alterations in fasting blood glucose, serum triglyceride, serum cholesterol, VLDL, HDL, LDL, SGOT, SGPT, Total Protein and body weight in diabetic rats. Thus the present study suggested the potential of *Asperagus racemosus* leaf in diabetes as well as related cardiovascular complications due to its antidiabetic and antihyperlipidemic properties.

Keywords: Streptozotocin, *Asperagus racemosus*, Antidiabetic, Antihyperlipidemic.

INTRODUCTION

Diabetes mellitus is a common endocrine disorder caused due to either deficiency in insulin production or due to ineffectiveness of the insulin produced. Such a deficiency results in impaired metabolism of glucose and other energy-1 yielding fuels like lipids and proteins. The metabolic disturbances contributes massively to most neurological, 2 cardiovascular, retinal and renal diabetic complications. The estimation that diabetes mellitus will affect more than 300 million people by the year 2025 shows the need for 3 improvement in the treatment aspect of this chronic disorder. Currently available pharmotheraphy for the treatment of diabetes mellitus include oral hypoglycemic agents and insulin. However these current drugs do not restore normal 4 glucose homeostasis and they are not free from side effects. Moreover due to high cost of allopathic drugs it is difficult to provide modern medical healthcare especially in developing countries. It is

therefore become necessary to make use of vast reserves of plant origins for medical purposes which will help to search effective as well as safer drug remedy for diabetes mellitus. Plant *Asperagus racemosus* belonging to family Asparagaceae is commonly known as Shatavari. The plant is native to India and grows in deciduous and evergreen forests and also in plains. Ripe fruits of *Asperagus curillus* cause abortion, tuberous roots with honey are given in dysuria, diabetes, and dysentery. The roots are bitter, sweet, emollient, cooling, nervine, tonic, constipating, opthalimic, anobnye, aphrodisiac. They are useful in nervous disorders, dyspepsia, tumours, scalding of urine, throat infections, tuberculosis, cough bronchitis and general debility. Roots are used externally to treat stiffness in the joints. The rhizome is a soothing tonic that acts mainly on the circulatory, digestive and respiratory system. In Unani system, the roots are used as laxatives, tonic, aphrodisiac, galactogogue,

and in disease of kidney and liver. We therefore subjected the ethanolic extract of leaf of *Asperagus racemosus* to preliminary phytochemical investigation which showed presence of alkaloids, tannins, flavonoids, saponins, glycosides and triterpenoids. The phytochemicals are indicative of its potential in the treatment of diabetes mellitus hence we undertook the present work to study the chronic antidiabetic effect and antihyperlipidemic effect of the bark extract in healthy and streptozotocin diabetic rats with the objective to focus on mechanism underlying the activity.

MATERIALS AND METHODS

Plant material and preparation of extract

The leaf of the plant *Asperagus racemosus* was collected from different regions of Mangalgi, vijayawada, after proper identification by an expert taxonomist Dr.A.Srinivas Rao, Department of botany, VRS&YRN Degree college, Chirala,A.P.,India. The leaves are shade dried and powdered and extracted successively with ethanol by soxhlet extraction. The extract were concentrated and dried in dessicator. Qualitative phytochemical tests were performed for phytoconstituents.

Experimental animals

Male albino Wistar rats, aged 4 months (body weight: 180 ± 10 g) were used for the present study, procured from Sainath enterprises, Hyderabad, India. The animals were housed in poly acrylic cages (38 cm \times 23 cm \times 10 cm) with not more than six animals per cage, at an ambient temperature of $18 \pm 2^{\circ}\text{C}$ with 12-h-light/12-h-dark cycle. Rats have free access to standard chow diet and water *ad libitum*. The Institutional animal Ethical Committee (IAEC) of KLR Pharmacy College, Paloncha, Khammam, A.P, India approved the animal experimental protocol.

Experimental induction of diabetes

Diabetes was induced by using streptozotocin as diabetogenic agent. Streptozotocin (65 mg/kg) was dissolved in ice cold citrate buffer (pH 4.3) immediately before use. The solution was injected intraperitoneally in the dose of 65 mg/kg in rats. 5 % glucose solution was administered orally for 24 hrs. to prevent mortality due to initial hypoglycemia induced by streptozotocin. After 72 hrs. of STZ injection, fasting blood glucose levels were tested using glucose oxidase-peroxidase reactive strips (Accu-check, Roche Diagnostics, USA). Rats showing fasting blood glucose more than 200 mg/kg were considered diabetic and used for further study.

Experimental Groups

Rats were divided in different groups as follows.

Group I: as normal control where rats received normal saline daily.

Group II: as diabetic control where diabetic rats received normal saline.

Group III: diabetic rats received 2.5 mg/kg of glipizide, an oral hypoglycemic agent.

Group IV: diabetic rats received 200 mg/kg ethanolic leaf extract of *Asperagus racemosus*.

Group V: diabetic rats received 400 mg/kg ethanolic leaf extract of *Asperagus racemosus*.

Experimental procedure

Blood glucose estimation

Fasting blood glucose levels were determined in all experimental rats initially to determine the diabetic status and thereafter every week during the 21 day study period. Blood was obtained by snipping tail of rat with the help of sharp razor and blood glucose levels were determined using glucometer (Ultra Touch Two, Johnson and Johnson). Each time the tail of the rat was sterilized with spirit.

Serum lipid profile estimation

At the end of 21 days, the blood samples were collected from retro orbital plexus of rats under anesthesia using a glass capillary tube, serum separated for determination of parameters like total cholesterol, HDL- cholesterol and triglycerides using commercially available kits (Span diagnostics), VLDL cholesterol and LDL- cholesterol were calculated using the Friedewald's formula.

$$\text{VLDL} = \text{Triglycerides} / 5$$

$$\text{LDL} = \text{Total cholesterol} - (\text{HDL-CH} + \text{VLDL-CH})$$

Statistical analysis

All results are expressed as the mean \pm SEM. The results were analysed for statistical significance by one way ANOVA followed by Dunnet's Multiple Test for comparison.

RESULTS AND DISCUSSION

The effect of STZ and plant extracts on blood glucose level is shown in Table 1. On repeated administration of ethanolic extract of *Asperagus racemosus* for 21 days, a sustained and significant ($p < 0.01$) decrease in blood glucose level of diabetic rats was observed in dose dependent manner as compared to diabetic control group. In diabetic rats blood glucose level was reduced by 30.3% and 45.79% at 200 and 400 mg/kg

doses of the extract respectively. The standard oral hypoglycemic drug glipizide (2.5mg/kg) showed more potent antidiabetic activity by reducing blood glucose level by 59.25% as compared to diabetic control group. However there was no significant effect of the extract on the blood glucose level of normoglycemic rats. shown in Table 2, STZ diabetic rats treated with extract showed significant ($p < 0.01$) reduction in the elevated levels of total cholesterol and triglycerides in diabetic rats. Chronic treatment of extract (400 mg/kg) and glipizide (2.5 mg/kg) reduced the LDL-cholesterol by 53.79% and 64.13% respectively as compared to diabetic control

group. Also the extract significantly ($p < 0.01$) improved the HDL-cholesterol level at 400 mg/kg. In addition the bark extract in dose of 400 mg/kg showed significant ($p < 0.01$) reduction in atherogenic index as comparable to glipizide (2.5 mg/kg).

As shown in Table 3, STZ diabetic rats showed significant ($p < 0.01$) reduction in body weight from 203.2 g to 158.7 g as compared to normal group. Oral administration of ethanolic extract of leaf of *Asperagus racemosus* (400 mg/kg) significantly ($p < 0.01$) and periodically improved the body weight after 21 days as compared to diabetic control.

Table 1: Effect of ethanolic extract on Blood glucose level in normal control and Streptozotocine induced diabetic rats

GROUPS	Blood glucose levels (mg/dl) Mean \pm SEM			
	1 st Day	7 th Day	14 th Day	21 st Day
Vehicle control	78.10 \pm 0.0570	71.3 \pm 0.28	70.41 \pm 0.29	71.95 \pm 0.48
Diabetic control	297.06 \pm 0.1230 ⁺	308.56 \pm 0.04 ⁺	293.6 \pm 0.43 ⁺	269.68 \pm 0.84 ⁺
Glipizide 2.5mg/kg	295.32 \pm 0.3702 ⁺	219.43 \pm 0.17 ⁺	159.65 \pm 0.69 ⁺	148.56 \pm 0.83 ⁺
200mg/kg EEAR	304.16 \pm 0.2137 ⁺	267.13 \pm 0.10 ⁺	240.25 \pm 0.18 ⁺	220.93 \pm 0.70 ⁺
400mg/kg EEAR	290.68 \pm 0.9662 ⁺	248.6 \pm 0.41 ⁺	209.43 \pm 0.53 ⁺	179.17 \pm 0.66 ⁺

value are expressed as mean \pm SEM (n=6)* $P < 0.001$, as compared to untreated control, ** $P < 0.001$, as compared to untreated control, One-way ANOVA followed by Dunnett's *t* tes.

Table 2: Effect of ethnolic extract on Lipid profile in normal control and Streptozotocine induced diabetic rats

GROUPS	TC(mg/dL)	TG(mg/dL)	VLDL(mg/dL)	LDL(mg/dL)	HDL(mg/dl)
Vehicle control	81.48 \pm 0.37	73.22 \pm 0.31	14.15 \pm 0.25	39.72 \pm 0.81	23.73 \pm 0.36
Diabetic control	222.53 \pm 0.94	114.46 \pm 0.60 ^a	22.52 \pm 0.52 ^{###}	132.58 \pm 0.93	20.91 \pm 0.33
Glipizide 2.5mg/kg	84.85 \pm 0.90	81.52 \pm 0.55	17.00 \pm 0.06 ^{##}	34.55 \pm 0.56	32.40 \pm 0.37
200mg/kg EEAR	132.9 \pm 0.48 ^{###}	95.02 \pm 0.05 ^{###}	20.04 \pm 0.03 ^{##}	68.48 \pm 0.51 ^{##}	40.22 \pm 0.37 ^{##}
400mg/kg EEAR	108.8 \pm 0.41	78.03 \pm 0.05	17.98 \pm 0.06 ^{##}	45.89 \pm 0.41 ^{##}	34.02 \pm 0.02 ^{##}

value are expressed as mean \pm SEM (n=6)* $P < 0.001$, as compared to untreated control, ** $P < 0.001$, as compared to untreated control, One-way ANOVA followed by Dunnett's *t* tes.

Table 3: Effect of ethnolic extract on Body weight in normal control and Streptozotocine induced diabetic rats

GROUPS	SGPT	SGOT	TOTAL PROTEIN	G6PD
Vehicle control	86.17 \pm 1.56	80.74 \pm 2.36	7.56 \pm 0.27	80.53 \pm 2.51
Diabetic control	139.1 \pm 2.29	146.0 \pm 1.40	8.94 \pm 0.05	69.4 \pm 3.38
Glipizide 2.5mg/kg	90.34 \pm 2.02	90.07 \pm 2.27	7.39 \pm 0.02	88.30 \pm 2.43
200mg/kg EEAR	101.4 \pm 1.90	84.01 \pm 1.74	8.52 \pm 0.05 ^{###}	64.73 \pm 4.37
400mg/kg EEAR	93.74 \pm 1.76 ^{###}	103.00 \pm 2.73	7.58 \pm 0.21	88.86 \pm 3.38

value are expressed as mean \pm SEM (n=6)* $P < 0.001$, as compared to untreated control, ** $P < 0.001$, as compared to untreated control, One-way ANOVA followed by Dunnett's *t* tes.

Table: 4 Effect of ethnolic extract on SGOT, SGPT, TOTAL PROTEIN, G6PD in normal control and Streptozotocine induced diabetic rats

Groups	BODY WEIGHTS (gm)			
	0 day	7 th day	14 th day	21 st day
Vehicle control	165.49 \pm 0.24	166.38 \pm 0.23	167.86 \pm 0.30	169.33 \pm 0.71
Diabetic control	162.03 \pm 0.17	154.19 \pm 0.28	144.3 \pm 0.30	129.56 \pm 0.34
Glipizide 2.5mg/kg	160.73 \pm 0.09	164.79 \pm 0.97	169.31 \pm 0.41 [*]	169.89 \pm 0.33
200mg/kg EEAR	158.47 \pm 0.12	159.83 \pm 0.71 ^{**}	169.33 \pm 0.30 ^{**}	158.83 \pm 0.66 ^{**}
400mg/kg EEAR	157.29 \pm 0.04	165.17 \pm 0.12	170.46 \pm 0.92 ^{**}	164.52 \pm 0.34

value are expressed as mean \pm SEM (n=6)* $P < 0.001$, as compared to untreated control, ** $P < 0.001$, as compared to untreated control, One-way ANOVA followed by Dunnett's *t* test.

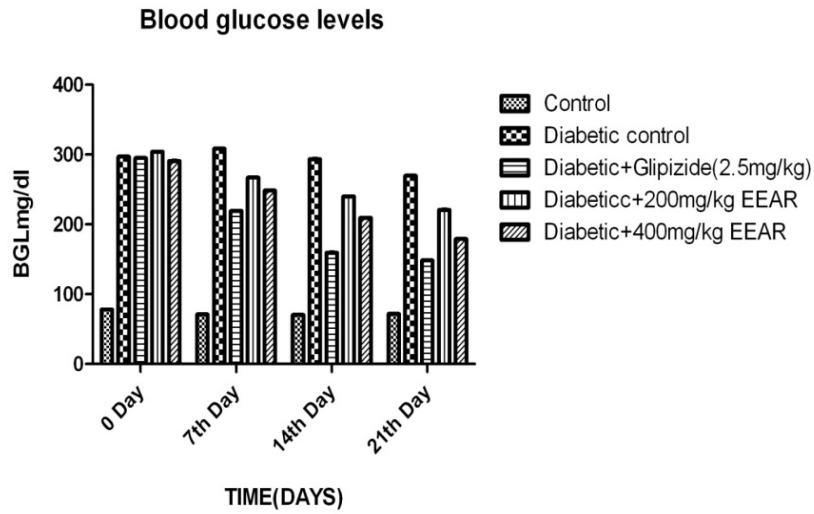


Fig. 1: showed of EEAR in Blood glucose levels on normal control and STZ induced diabetic rats. Values are expressed as Mean ± SD of 6 animals in each group.

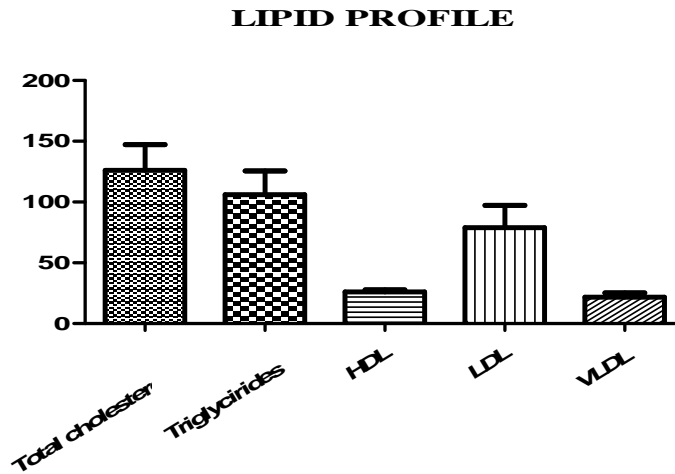


Fig. 2: showed effect of EEAR on Lipid profile in normal control and STZ induced diabetic rats. Values are expressed as Mean ± SD of 6 animals in each group

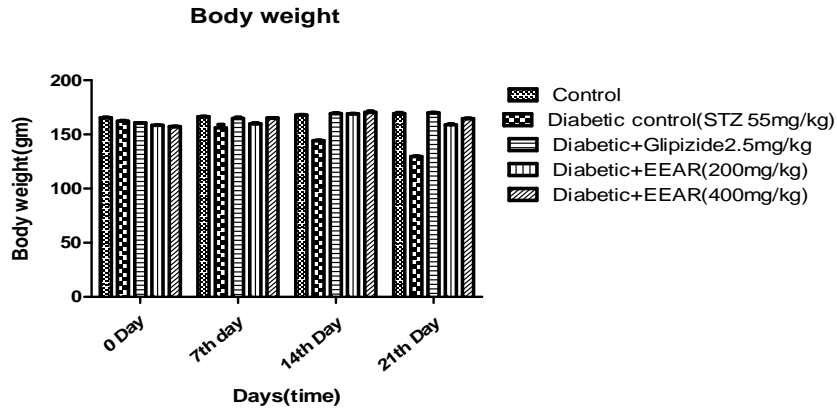


Fig. 3: showed Effect of EEAR on initial Blood glucose levels in normal control and STZ induced diabetic rats. Values are expressed as Mean ± SD of 6 animals in each group

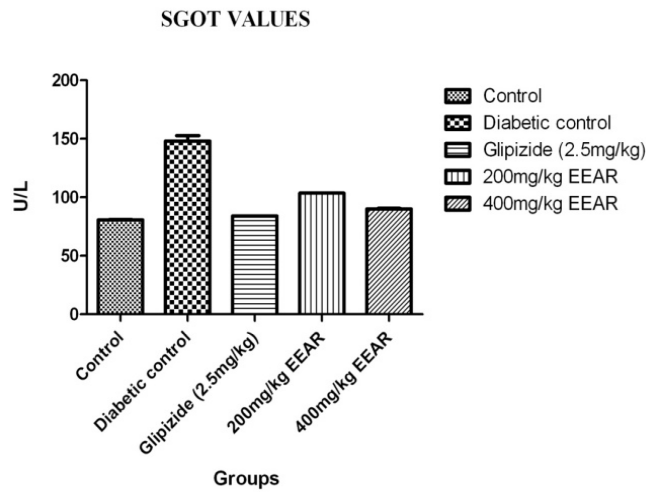


Fig. 4: showed effect of ethanol extract on SGOT in normal control and STZ induced diabetic rats. Values are expressed as Mean ± SD of 6 animals in each group

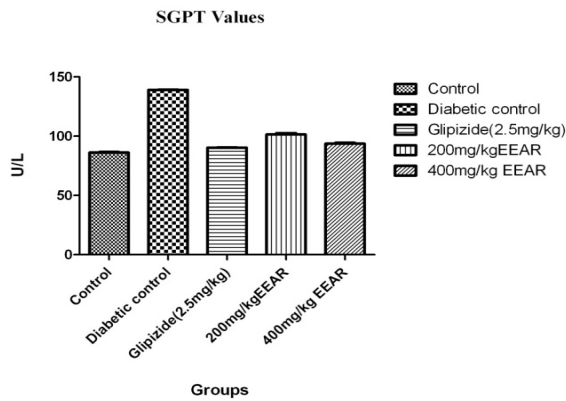


Fig. 5: showed effect of ethanol extract on SGPT in normal control and STZ induced diabetic rats. Values are expressed as Mean ± SD of 6 animals in each group

CONCLUSION

The present study showed that ethanolic extract of *Asperagus racemosus* significantly reduced elevated blood glucose level in STZ diabetic rats without showing any hypoglycemic effect in normal rats. Since STZ effectively destroys pancreatic beta cells and causes persistent hyperglycemia, the mechanism of action of *Asperagus racemosus* might involve actions other than pancreatic beta cells insulin release or secretion. The antidiabetic effect of the extract could be due to increased utilization of glucose by peripheral tissues, improved sensitivity of target tissues for insulin or it may be due to improved metabolic regulation of glucose. Our findings that *Asperagus racemosus* leaf significantly reduced serum triglyceride levels in STZ diabetic rats support its long term use not only for better control of blood glucose but also for normalization of disturbances in lipid metabolism which may prevent further predisposition of the patients to cardiovascular complications. Thus the present study showed that leaf of *Asperagus racemosus* possesses antidiabetic and antihyperlipidemic effects in STZ diabetic rats. The antiatherogenic potential of the leaf extract indicates its usefulness not only in diabetes mellitus but also in long term complications associated with diabetes mellitus. However comprehensive research is required to identify the active constituents responsible for this effect.

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