ANTIDIABETIC EFFECT OF *ANTIGONON LEPTOPUS* HOOK & ARN LEAF ON STREPTOZOTOCIN - INDUCED DIABETIC RATS

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Summary

The present study was aimed to evaluate the fractions of *Antigonon leptopus* Hook & Arn leaf for their antidiabetic potential on streptozotocin-induced diabetes in neonatal rats. The methanolic extract of the leaves of *A.leptopus* (MAL) had showed significant oral glucose tolerance at 200 mg/kg body weight, which was conducted in our labs. Oral administration of toluene, ethyl acetate, and butanone fractions of *A.leptopus* leaf (TMAL, EAMAL, BNMAL) at 50 and 100 mg/kg b.w significantly reduced the fasting blood glucose level in diabetic rats. Among these fractions, EAMAL was found to be more effective. Further, the effects of EAMAL on fasting blood sugar level and serum biochemical analysis in Streptozotocin - induced diabetic rats were investigated. The results revealed that EAMAL exhibited significant antidiabetic activity and substantiating the traditional medicinal claim of the leaves of *A.leptopus* in the treatment of diabetes.

Key Words: Antidiabetic activity, *Antigonon leptopus*, Streptozotocin.

Introduction

Antigonon leptopus (Family-Polygonaceae) is native to Mexico and commonly found in tropical Asia, Africa, Caribbean and in America. In India, it is most common in the upper Ganges plains and Himalayan regions, which is a climbing plant that flourishes throughout the lowlands, beside streams and gullies as well as on dry and sandy heaps along the coast. The vines of this plant regularly display a pleasing veil of green, followed by spray of pink flowers.

Traditionally the leaves of *A.leptopus* are used to reduce swelling and to treat diabetes, hypertension, and menstrual pains. A tea prepared from the leaves of *A.leptopus* used for diabetes (1). The vine is used to treat cough and throat constrictions. In Chinese traditional medicine, it is used for the treatment of nephritis, hepatitis and colitis (2).

Chemical investigations of the aerial parts of *A.leptopus* revealed that the leaves and flowers contain flavonoidal glycosides viz quercetin-3-rhamnoside and quercetine-3-o-glucoronide in the leaves of *A.leptopus* (3). Flavonoidal compounds viz quercetin, rhamnetin, quercetin - 3-o-β-D-glucopyranoside and a new anthraquinone glycoside: 1, 5-dihydroxy – 3- methyl anthraquinone-8-o-(4-o-α –L- arabino furanosyl) - β-D-glucopyranoside, quercetin-3-o- rhamnosyl rhamnoside and two anthocyanins viz, pelargonin and malvin were isolated from the acidic (1% HCl) methanolic extract of fresh flowers (4,5,6). A new anthraquinoid pigment 1, 6, 8-trimethoxy-3- propanoyl-anthraquinone was isolated from the methanolic extract of flowers (7). The polyphenols viz 4-hydroxyl cinnamic acid, quercetin - 3-rhamnoside and kaempferol-3-glucoside was isolated from the methanolic extract of aerial parts of *A. leptopus* and inhibited lipid peroxidation (LPO) and COX-1 and COX-2 enzyme activity (8). The methanolic extract of *A.leptopus* root has analgesic and anti-inflammatory properties, which was reported from our lab (9) and the methanolic extract of vine has antithrombin activity (10).

In view of the folklore use in the treatment of diabetes and the presence of flavonoidal glycosides of the leaves of *A.leptopus*, the present study was undertaken to assess the efficacy of this indigenous herb for antidiabetic activity in rats.

Materials and Methods

Chemicals: Glibenclamide is a generous gift from Orchid laboratories, Chennai, India. Streptozotocin (STZ) was purchased from Sigma-Aldrich Company, Germany. Glucose, serum glutamate pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total protein, creatinine, triglycerides, total cholesterol levels were studied by 'Merck Micro lab 300' analyzer by using Merck analytical kits. All other chemicals used were of analytical grade.

Plant material: Fresh leaves of *A.leptopus* were collected in the month of August 2009 from Warangal, Andhra Pradesh, India after the authentification by Prof. V. S. Raju, Department of Botany, Kakatiya University, Warangal. A

Voucher Specimen (KU/UCPSC/No.43) has been kept in the herbarium of University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India for future reference.

Preparation of the extract: The leaves of *A.leptopus* were shade dried and made into coarse powder, subjected for maceration process with methanol at room temperature. After exhaustive extraction, the methanolic extract was concentrated under reduced pressure at 50^0 – 55^0 C and stored in a vacuum desiccator. The concentrated methanolic extract was suspended in water and fractionated with toluene, ethyl acetate, butanone in succession. The yield of fraction was found to be 27g of toluene fraction, 6.3g of ethyl acetate fraction, and 3g of butanone fraction. Fine suspensions of the fractions were prepared with 5% gum acacia was used for the experiments.

Animals: Wister albino rats weighing between 160-180 g and mice weighing between 22-25 g were purchased from M/s Mahaveer enterprises, Hyderabad and used for present study. They were housed in polypropylene cages and fed with standard diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. All the experiments on animals were conducted after obtaining permission from Institutional Animal Ethical Committee.

Acute toxicity study: Acute toxicity study was carried out according to the method described in the literature (11)**.** TMAL, EAMAL, BNMAL were suspended in 5% gum acacia solution administered orally to albino mice of either sex (22-25 g) in doses of 100, 200, 400, 800, 1000, 1200, 1400, 1800, 2000 mg/kg b.w. The animals were observed continuously for any change in autonomic 'or' behavioral responses for first few hours and later at 24 h interval for a period of 48 h and noted the mortality if any.

Effect of methanolic fractions of *A.leptopus* leaf on fasting blood glucose level in streptozotocin - induced type2 diabetic rats

Streptozotocin was dissolved in citrate buffer (pH 4.5) and injected to 48± 2 h old neonatal rats i.p at a dose of 90 mg/kg b.w. The diabetic rats (glucose level > 150 mg/dl) were separated after 12 weeks of streptozotocin administration and divided into 8 groups of six animals each (12). Group I served as diabetic control, received vehicle (5% gum acacia), group II served as standard, received glibenclamide 10 mg/kg b.w , group III, IV, V, VI, VII and VIII received TMAL, EAMAL and BNMAL respectively (50 and 100 mg/kg b.w of each

fraction). Blood samples of the rats were collected just before and 2, 4, 6, 8, 12 and 24 h after administration of the vehicle, standard and methanolic fractions of *A.leptopus* test samples and were analyzed for blood glucose content by using glucose oxidase method (13).

Sub acute treatment: The diabetic rats (glucose level > 150 mg/dl) were divided into 3 groups of six animals each. Group I served as diabetic control received 5% gum acacia, group II served as standard received glibenclamide (10 mg/kg b.w) and group III received EAMAL (100 mg/kg b.w). EAMAL was administered once daily for 28 days. During the study period, the body weight of the animals and blood glucose level were recorded after 7, 14, 21 and 28th day of the treatment. Cholesterol, triglycerides, insulin, SGPT, ALP, creatinine and total proteins level in serum were estimated at the initial and after 28 days of the treatment.

Statistical analysis: All the values were expressed as mean ± SD. The data was statistically evaluated using one way analysis of variance (ANOVA) followed by Dunnetts t-multiple comparison test using Graph pad Prism 3 computer software. P value of 0.05 or less was considered as significant.

Results

Acute toxicity study

No adverse effects and no mortality of the animals were observed during the period of study up to the dose 2000 mg/kg. Hence, the two doses i.e. 50 and 100 mg/kg of TMAL, EAMAL, and BNMAL were selected for the study.

Effect of methanolic fractions of *A.leptopus* leaf on fasting blood glucose

The results of the study are shown in Table 1. All the three fractions (TMAL, EAMAL, BNMAL) exhibited significant (p<0.05) reduction in fasting blood glucose level at the two test doses i.e. 50 and 100mg/kg b.w. after 2 h of administration and continued the effect up to 12 h. TMAL at both the test doses exhibited maximum reduction in blood glucose level after 6 h (36.3%, 39.7%) whereas EAMAL and BNMAL showed the same effect after 4 h of administration (45.0%, 57.0% and 23.7%, 36.7%). However, EAMAL at 100 mg/kg b.w was found to be more effective (p<0.01) in reducing the fasting blood glucose level in streptozotocin - induced diabetic rats, which is well comparable to that of the effect of reference drug, glibenclamide (p<0.01).

Table-1. Effect of methanolic fractions of *Antigonon leptopus* leaves on blood glucose level in streptozotocin - induced diabetic rats

		Blood glucose level (mg/dl) at different hours after the treatment						
Group	**Dose (mg/kg)**	**0 hr**	**2 hr**	**4hr**	**6hr**	**8 hr**	**12 hr**	**24 hr**
Diabetic control	---	159.67± 28.48	156.33± 25.24	152.66± 29.47	154.83± 29.70	156.83± 24.86	158.16± 25.46	157.00± 34.40
Glibenclamide	10	202.5± 12.60	133.5± 37.11*	101.33± 31.56**	127.5± 28.33**	150.16± 19.33*	151.83± 36.94*	180.66± 18.07
TMAL	50	154.5± 1.70	114.83± 3.02*	108.33± 2.74**	98.33± 2.35**	112.66± 3.03*	129.00± 4.20*	141.33± 3.39
TMAL	100	156.66± 4.22	112.83± 5.39*	100.66± 5.76**	94.16± 6.89**	125.33± 37.17*	127.16± 6.28*	147.16± 3.67
EAMAL	50	202.33± 28.56	132.83± 26.39*	114.16± 25.74**	121.8± 24.00**	125.83± 28.97*	120.00± 28.21*	144.5± 29.14
EAMAL	100	177.66± 29.78	100± 11.56*	76.33± 27.81***	80.16± 18.99***	99.50± 13.13*	110.00± 21.18*	127.5± 29.86
BNMAL	50	185.5± 3.44	148.33± 4.74*	141.16± 3.65*	144.5± 4.57*	148.66± 1.69*	159.33± 5.49*	169.00± 4.16
BNMAL	100	177.33± 3.44	146.83± 10.20*	112.33± 6.31*	137.33± 8.91*	140.5± 10.14*	142.67± 9.55*	148.66± 8.19

Statistically significant * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to diabetic control at the respective time point; Data represented as mean ± SD.
TMAL: Toluene fraction of *Antigonon leptopus*, EAMAL: Ethyl acetate fraction of *Antigonon leptopus*, BNMAL: Butanone fraction of *Antigonon leptopus.*

Effect of EAMAL on different parameters in sub-acute study (28 days)

Body weight

There was a gradual diminution in body weight of animals in diabetic control group**.** The animals of extract (EAMAL) treated and reference drug treated groups showed a gradual increase in the body weight after 7days of treatment. The increase in the body weight was observed till the end of the study (28 days).The significant ($p<0.01$) effect of EAMAL (100 mg/kg b.w) on body weight of the animals was comparable to that of the reference drug, glibenclamide (10 mg/kg b.w). The results are shown in Table 2.

Biochemical Changes

EAMAL and the reference drug, lowered the blood glucose level ($p<0.01$) gradually after 7 days of the treatment and continued the effect up to the end of the study ($p<0.01$). The maximum effect was observed after 28 days showing 47.4% reduction in blood glucose level. The significant antihyperglycemic effect of the fraction was well comparable to that of the reference drug, glibenclamide (10 mg/kg b.w) showed 44.0% reduction at each time interval of the study. The results are shown in Table 2.

EAMAL at 100 mg/kg b.w. showed a significant ($p<0.01$) effect on cholesterol, triglycerides, SGPT, ALP and creatinine level in serum by reducing their elevated level while increasing the diminished serum insulin and total protein levels in streptozotocin induced diabetic rats, and it was comparable to that of the reference drug. The results are shown in Table 3.

Table -2: Effect of EAMAL on different parameters in streptozotocin - induced type 2 diabetic rats (Sub acute study)

Group	Body weight(g)					Blood glucose (mg/dl)				
	Days of treatment					Days of treatment				
	1	7	14	21	28	1	7	14	21	28
Diabetic control	150.3 ± 4.0	146.8 ± 2.9	142.2 ± 2.5	142.2 ± 1.8	140.0 ± 1.2	202.1 ± 8.6	209.17± 12.4	217.8 ± 21.2	241.1 ± 19.6	258.0 ± 23.4
Glibenclamide (10mg/kg)	155.4 ± 5.5	160.5 ± 5.2	161.0 ± 5.6	161.6 ± 5.0	164.0 ± 4.6*	181.6 ± 17.8	168.4 ± 21.7*	148.8 ± 26.9**	131.0 ± 14.0**	101.8 ± 8.9**
EAMAL (100mg/kg)	155.5 ± 6.6	157.1 ± 6.2	158.8 ± 7.6	160 ± 5.2	164.6 ± 4.4*	198.1 ± 48	185.5 ± 34.9*	165.5 ± 27**	147.8 ± 27.1**	104.6 ± 9.4**

Statistically significant * p<0.05, ** p<0.01 compared to diabetic control at the respective time point; Data represented as mean± SD.

EAMAL: Ethyl acetate fraction of *Antigonon leptopus.*

Table-3. Effect of EAMAL on different parameters in streptozotocin - induced type 2 diabetic rats (Sub acute study)

Group	Serum Cholesterol (mg/dl)		Triglycerides (mg/dl)		SGPT (IU/L)		ALP (IU/L)		Creatinine (mg/dl)		Insulin (µIU/ML)		Total protein (mg/dl)	
	1st day	28th day	1st day	28th day	1st day	28th day	1st day	28th day	1st day	28th day	1st day	28th day	1st day	28th day
Diabetic control	131.73 ± 4.5	122.42 ± 6.7	168.5 ± 22.6	168.5 ± 22.6	77.36± 2.0	77.4± 4.5	301.7 ± 15.1	350.9 ± 27.2	1.9 ± 0.15	2.26 ± 0.3	8.98 ± 0.4	8.6 ± 0.5	4.3 ± 0.4	4.2 ± 0.4
Glibenclamide	110.83 ± 10.4	83.0 ± 5.2**	168.6 ± 9.5	131.0 ± 8.4**	72.6± 0.3	26.13± 3.9**	294.5 ± 27.0	162.5 ± 13.8**	2.2 ± 0.42	1.3 ± 0.2**	8.8 ± 0.9	16.9 ± 0.9**	5.6 ± 0.54	7.5 ± 0.5**
EAMAL	119.43 ± 6.4	79.43 ± 4.1**	178.5 ± 6.6	138.3 ± 9.6**	76.01± 0.3	31.16± 4.13**	315.5 ± 22.5	152.0 ± 18.5**	2.0 ± 0.5	1.18 ± 0.2**	9.03 ± 0.4	15.5 ± 1.5**	4.0 ± 0.48	7.0 ± 0.4**

Statistically significant * p<0.05, ** p<0.01 compared to diabetic control at the respective time point; Data represented as mean ± SD.
EAMAL: Ethyl acetate fraction of *Antigonon leptopus.*

Discussion

The results of the study indicate that all the three fractions (TMAL,EAMAL,BNMAL) are non toxic up to a dose of 2000 mg/kg b.w. and have significant ($p<0.01$) anti hyperglycemic activity in streptozotocin - induced diabetic rats. Among the three fractions, EAMAL at 100 mg/kg b.w. was showed significant ($p<0.01$) effect on fasting blood glucose level after 4 h of administration and comparable with glibenclamide (10 mg/kg b.w.), the reference hypoglycemic drug of sulphonylurea type (14). In the treatment of 48 h old rats with streptozotocin produces a relatively moderate increase and decrease in fasting blood glucose level and insulin level respectively i.e. a rat model of type2 diabetes, (15) and the antihyperglycemic effect of EAMAL in the study could be explained in terms of potentiating glucose induced insulin secretion.

In the sub acute study, administration of EAMAL at 100 mg/kg b.w. brought about beneficial changes on body weight as well as on different serum biochemical parameters. A significant improvement in body weight indicates the ability of EAMAL to prevent loss of body weight in diabetic rats (16). As the effect was quite similar with that of reference drug, glibenclamide reveals that EAMAL do not have any effect on degradation of depot fat and it can maintain the bodyweight and this could be due to its ability to reduce hyperglycemia. The significant ($p<0.01$) antihyperglycemic effect of EAMAL observed in this study was supported by significant changes in other serum parameters such as decrease in cholesterol, triglycerides, SGPT, ALP, creatinine and increase in insulin and total protein.

Hypercholesterolemia and hypertrygliceredemia have been reported to occur in streptozotocin - induced diabetic rats. In insulin deficient subjects, it fails to activate the enzyme lipoprotein lipase and causes hypertriglyceredemia (15). Hence, it is possible that the mechanism of reduction of serum lipid levels with EAMAL may be through insulin release or by enhancing insulin sensitivity in the tissues, which was also evident from the increase in serum insulin level. The effect of EAMAL was similar as that of the reference drug, glibenclamide, insulin secretogogue

indicating that the antihyperglycemic effect of EAMAL may be due to its stimulatory effect on insulin secretion (17). The diminution of serum GPT, ALP and creatinine level with EAMAL further strengthens the antidiabetogenic effect of EAMAL as the hepatoprotective enzymes (SGPT, ALP) and creatinine levels are elevated in streptozotocin - induced diabetic rats due to damage to the structural integrity of the liver and degenerative condition in the kidney (18,19).

Thus, it can be concluded that the leaves of *Antigonon leptopus* possess significant antidiabetic properties, which substantiates the traditional claim of the leaves for treatment of diabetes. The ethyl acetate fraction of methanolic extract of leaves of *Antigonon leptopus* (EAMAL) has antihyperglycemic potential without any toxic effects and thus it could be beneficial in the management of type 2 diabetes. Therefore, further studies on EAMAL are in progress to isolate and characterize the active constituents from it and to elucidate the exact mechanism of antidiabetic activity.

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