Research Paper

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Antidiabetic and Antihyperlipidamic Activity of the Ethanolic leaf Extract of *Glycosmis pentaphylla* against Alloxan Induced Diabetes in Albino Rats

Pharma

KEYWORDS : Glycosmis pentaphylla, alloxan-induced, anti diabetic activity, antihyperlipidemic, glipizide.

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ABSTRACT

Leaf powder of Glycosmis pentaphylla was subjected to hot extraction (soxhlet) with ethanol. After preliminary phytochemical investigation ethanolic extract of the leaves of Glycosmis pentaphylla was evaluated for anti diabetic and antihyperlipidemic activity against alloxan-induced diabetes in albino rats. The ethanolic extracts showed significant activity as compare to standard glipizide.

INTRODUCTION:

Glycosmis pentaphylla (Rutaceae) is commonly called as orange berry, Juice of leaves, is a shrub or small (1.5–5 m) tree widely distributed from India, Malaysia and southern China to the Philippine Islands where it occurs in tropical forests at low altitudes. It has been used as a folk medicine in the treatment of fever, liver complaints and certain other diseases (Sastri, 1956). In Hindu medicines, it has been used traditionally in bilious complaints, cough, worms, jaundice and fever. This drug has been proved by CCRH, monograph of which has also been published. This drug is proved to be effective in bilious complaints like nausea, vomiting, bitter taste in the mouth, heart burn with desire for lime juice, which ameliorates and gastric symptoms are aggaravated by eating, Migraine-pain in temples. Juice of leaves is used in fever,

liver complaints and as a vermifuge, while leaves are considered good antidote for eczema and other skin troubles14,15,16.Here the experiment of leaves has been evaluated for anti diabetic activity and antihyperlipidemic against alloxan induced model using standard drug glibenclamide.

MATERIALS AND METHODS:

The leaves of Glycosmis pentaphylla (Retz) DC were collected from the local forest areas around the Tirupati, A.P, India, and authenticated by the botanist Dr. Madhava Chetty K, Department of botany, Sri Venkateswara University, Tirupati, AP, India. All the solvents and chemicals were of analytical grade and procured from Merck Ltd., Ambernath and Alloxan monohydrate was obtained from SD Fine Chemicals Pvt. Ltd. Biosar,

PREPARATION OF EXTRACT:

The leaves were dried under shade and then powdered and stored in airtight container. The shade dried leaves of Glycosmis pentaphylla was powdered (1000g) and extracted with ethanol (99.99%), in 1:3 ratios in soxhlet apparatus exhaustively for 20-24 hours. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using flash evaporator and a semisolid extract was obtained, which was used in the study.

ANIMALS:

Male Sprague Dawley rats, aged 4 months(body weight: 160 ± 10g) were used for the present study, procured from Mahaveer enterprises, Hyderabad, India. The animals were housed in poly acrylic cages (38 cm × 23 cm × 10 cm) with not more than six animals per cage, and maintained under standard conditions (27±2ºC, relative humidity 44 - 56% and light and dark cycles of 10 and 14 hours respectively) and fed with standard rat diet and purified drinking water ad libitum for 1 week before and during the experiments. All experiments and protocols described in present study were approved by the Institutional Animal Ethical Committee (IAEC) of KLR Pharmacy College, Paloncha, Khammam (No.1516/PO/a/11/CPCSEA).

ANTIDIABETIC ACTIVITY

Hyperglycemia / diabetes was induced by single Intraperitonial injection of freshly prepared aqueous solution of alloxan monohydrate 150 mg/kg, to overnight fasted rats. After 48 hrs of alloxan injection, the animals which did not developed hyperglycemia i.e glucose level >200mg/dl, were rejected/replaced with new animals. Immediately after confirmation of diabetes, rats were classified into five groups of six rats each. Standard drug used for treatment, Glipizide, 10 mg/kg, ethanolic test extract were prepared, 400mg/kg and 800mg/kg in 2% Carboxy Methyl Cellulose (CMC) and were given orally. Taking six rats in each five groups did evaluations of antidiabetic effect. Treatment was continued for 28 consecutive days, with twice a day dose (morning and evening). Before the treatment (0day) and at the end of 7th,14th 21st and 28th day, blood samples were collected from the tip of the tail of each rat under mild ether anesthesia in 1 ml Eppendrof tubes containing 50µl of anticoagulant (heparin)1,2,3,4. Serum separated by centrifugation of blood at 4000rpm for 10mins was subjected for estimating glucose by Glucose oxidase method using semi auto- analyzer. It was done with 1 ml of blood withdrawn on 28th day, from all five groups rats (normal, diabetic control, extracts of 400, 800mg/kg and standard treated) and stored in a refrigerator until analyzed. And the serum was subjected for the estimation of triglyceride (TGL), HDL, LDL, VLDL and total cholesterol level 5,6,7,8,9,10.

RESULTS AND DISCUSSION:

Diabetes induction caused significant (P<0.001) hyperglycaemia in all diabetic groups (Table 1). Oral administration of the extract and Glipizide for 28 days significantly (P<0.001) lowered the hyperglycaemia of the experimental groups, when compared to diabetic control group. Fasting blood glucose levels of untreated diabetic rats were significantly higher than those in normal rats. Over production of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental bases of hyperglycemia in diabetes mellitus. The fasting blood glucose of the groups treated with plant extract 400mg/kg, 800mg/kg (body weight) lowered the glucose level from 191 mg/dl to 152 mg/dl and 197 mg/dl to 138 mg/dl and Glipizide from 192 mg/dl to 116 mg/dl representing 20.67%, 29.94% and 39.32% reductions respectively. The effect on the fasting blood glucose is dose dependent (Table 1 & 2). The proposed mechanism of action may be by promoting regeneration of β-cells or by protecting the cells in pancreas from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action and further effect β-cells to release insulin and activate the insulin receptors to absorb the blood sugar. Regeneration of islet β-cells following

Table.No:1 The effect of 28th day treatment with ethanolic extract of Glycosmis pentaphylla on blood glucose levels (mg/dl) in Alloxan induced diabetes in rats.

Groups	Blood glucose levels (mg/dl) Mean ± SEM			
	0 min	30 min	60 min	120 min
I- Normal control	91.33 ± 3.50	111.50 ± 4.10	102.33 ± 3.87	92.16 ± 3.46
II- Diabetic control (Alloxan130mg/kg)	292.66 ± 7.75 a	451.33 ± 17.13 a	423.50 ± 17.21a	392.33 ± 16.11a
III- Diabetic + STD (Glipizide10mg/kg)	116.50 ± 8.20 c	209.00 ± 8.20 a	159.16 ± 7.03 b	121.66 ± 7.03 ns
IV- Diabetic + GP(400mg/kg)	159.50 ± 4.10 a	259.00 ± 4.15 a	226.83 ± 3.91 a	191.16 ± 2.92 a
V- Diabetic + GP(800mg/kg)	139.83 ± 7.94 a	229.83 ± 14.30 a	195.83 ± 14.30 a	158.50 ± 11.29 a

All values given in average blood glucose levels (mg/dl) shown are Mean ± SEM and n=6, Data was analyzed using one way ANOVA and Dunnett's t test. a=P<0.001,b=P<0.01

Groups	Blood glucose levels (mg/dl) Mean ± SEM				
	1st Day	7th Day	14th Day	21st Day	28th Day
I- Normal control	87.50 ± 3.06	84.83 ± 2.00	91.83 ± 3.59	88.83 ± 3.13	90.33 ± 2.60
II- Diabetic control (Alloxan 130mg/kg)	198.16 ± 3.47 a	237.66 ± 3.38 a	296.33 ± 7.61a	315.50 ± 8.86 a	324.00 ± 9.96 a
III- Diabetic + STD (Glipizide 10mg/kg)	192.00 ± 7.40 a	165.50 ± 3.01a	142.50 ± 7.75 a	122.16 ± 3.79 a	116.50 ± 4.42 b
IV- Diabetic + GP (400mg/kg)	191.83 ± 6.87 a	185.50 ± 6.85 a	171.16 ± 5.31a	161.50 ± 4.97 a	152.16 ± 2.96 a
V- Diabetic + GP (800mg/kg)	197.00 ± 6.24 a	181.66 ± 7.41a	165.50 ± 6.54 a	147.33 ± 4.66 a	138.00 ± 3.68 a

Table.No:2 The effect of ethanolic extract of Glycosmis pentaphylla on oral glucose tolerance in normal and alloxan induced diabetic rats.

All values given in average blood glucose levels (mg/dl) shown are Mean ± SEM, and n=6, Data was analyzed using one way ANOVA and Dunnett's t test.a=P<0.001,b=P<0.0

Table.No:3 Showing triglyceride levels (mg/dl) (Mean ± SEM) (n=6) in different groups of rats compared to normal control.

Groups	Triglycerides (mg/dl) ± SEM		
	1st day	14th day	28th day
I- Normal control	67.37 ± 0.56	68.15 ± 0.95	69.13 ± 0.81
II- Diabetic control (Alloxan 130mg/kg)	70.50 ± 0.64**	114.67 ± 1.56***	170.07 ± 1.09***
III- Diabetic + STD (Glipizide 10mg/kg)	70.51 ± 0.62**	86.64 ± 1.11***	82.05 ± 0.60***
IV- Diabetic + GP(400mg/kg)	69.78 ± 0.75*	89.24 ± 1.23***	81.11 ± 0.56***
V- Diabetic + GP(800mg/kg)	70.24 ± 0.60*	90.17 ± 0.90***	78.12 ± 1.02***

All values given in average triglyceride levels (mg/dl) shown are Mean ± SEM and n=6 Data was analyzed using one way ANOVA and Dunnett's t-test. *P<0.05, **P<0.01, ***P<0.00

Table.No:4 Showing total cholesterol levels (mg/dl) (Mean ± SEM) (n=6) in different groups of rats compared to normal control.

Group	Total Cholesterol (mg/dl) Mean ± SEM		
	1st day	14th day	28th day
I- Normal control	73.05 ± 1.00	72.78 ± 0.89	74.14 ± 1.42
II- Diabetic control (Alloxan 130mg/kg)	75.20 ± 0.69 ns	119.70 ± 1.29***	139.04 ± 0.78***
III- Diabetic + STD (Glipizide 10mg/kg)	74.59 ± 0.82 ns	92.70 ± 1.10***	86.31 ± 0.83***
IV- Diabetic + GP(400mg/kg)	78.26 ± 0.95***	96.59 ± 2.33***	86.90 ± 0.52***
V- Diabetic + GP(800mg/kg)	75.70 ± 0.57 ns	96.73 ± 0.76***	83.60 ± 1.16***

All values given in average total Cholesterol levels (mg/dl) shown are Mean ± SEM and n=6, Data was analyzed using one way ANOVA and Dunnett's t-test. *P<0.05, **P<0.01, ***P<0.001,ns=non significance.

Table.No:5 Showing HDL-c levels (mg/dl) (Mean ± SEM) (n=6) in different groups of rats compared to normal control.

Groups	HDL-c (mg/dl) Mean ± SEM		
	1st day	14th day	28th day
I- Normal control	30.16 ± 0.58	28.82 ± 0.66	31.06 ± 0.64
II- Diabetic control (Alloxan 130mg/kg)	29.37 ± 0.52 ns	19.34 ± 0.26***	17.18 ± 0.36***
III- Diabetic + STD (Glipizide 10mg/kg)	29.36 ± 0.68 ns	22.00 ± 0.47***	24.17 ± 0.43***
IV- Diabetic + GP(400mg/kg)	29.07 ± 0.80 ns	21.02 ± 0.59***	24.19 ± 0.43***
V- Diabetic + GP(800mg/kg)	29.52 ± 0.34 ns	19.30 ± 0.62***	24.35 ± 0.34***

All values given in average HDL-c level (mg/dl) shown are Mean ± SEM and n=6

Data was analyzed using one way ANOVA and Dunnett's t -test. *P<0.05, **P<0.01, ***P<0.001ns=non significance.

Table.No:6 Showing LDL-c levels (mg/dl) (Mean ± SEM) (n=6) in different groups of rats compared to normal control.

Groups	LDL-c (mg/dl)Mean ± SEM		
	1st day	14th day	28th day
I- Normal control	32.46 ± 0.89	32.62 ± 1.13	34.79 ± 1.19
II- Diabetic control (Alloxan 130mg/kg)	35.34 ± 0.58 ns	97.25 ± 1.12***	129.64 ± 0.78***
III- Diabetic + STD(Glipizide 10mg/kg)	34.20 ± 0.93 ns	50.66 ± 0.73***	47.45 ± 1.09***
IV- Diabetic + GP(400mg/kg)	35.93 ± 0.79*	59.00 ± 1.70***	44.52 ± 0.58***
V- Diabetic + GP(800mg/kg)	35.51 ± 0.77 ns	59.45 ± 0.84***	44.00 ± 0.94***

All values given in average LDL-c levels (mg/dl) shown are Mean ± SEM and n=6.

Data was analyzed using one way ANOVA and Dunnett's t-test. *P<0.05, **P<0.01, ***P<0.001,ns=non significance.

Table.No:7 Showing SGPT (U/L), SGOT (U/L) levels (Mean ± SEM) (n=6) in different groups of rats compared to normal control.

Groups	Mean ± SEM	
	SGPT (U/L)	SGOT (U/L)
I- Normal control	89.16 ± 1.07	116.33 ± 2.98
II- Diabetic control (Alloxan 130mg/kg)	144.00 ± 2.11 a***	149.66 ± 1.40 a***
III- Diabetic + STD(Glipizide 10mg/kg)	99.33 ± 2.21 b***	120.16 ± 2.27 b***
IV- Diabetic + GP(400mg/kg)	127.66 ± 1.90 a***	139.16 ± 1.74 a***
V- Diabetic + GP(800mg/kg)	111.83 ± 1.66 a***	130.00 ± 2.73 a***

All values given in average SGPT,SGOT shown are Mean ± SEM and n=6.Data was analyzed using one way ANOVA and Dunnett's t-test. *P<0.05, **P<0.01, ***P<0.001, a= when compared with normal group,b= when compared with diabetic control.

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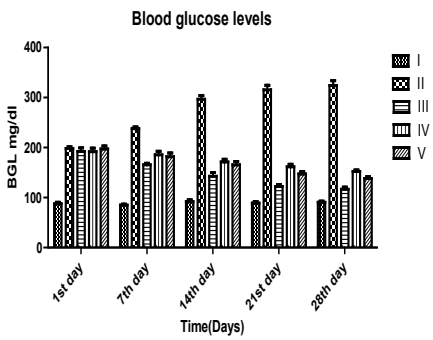
Table.No:8 The effect of 28th day's treatment with ethanolic extract of plant Glycosmis pentaphylla on body weight (g) after alloxan induced diabetes in rats.

GROUPS	BODY WEIGHTS (gm)		% of increased body wt
	Initial	Final	
I- Normal control	170.33 ± 3.74	188.83 ± 3.67	9.98
II- Diabetic control (Alloxan 130mg/kg)	176.16 ± 1.55 ns	126.83 ± 2.52 a***	-38.89
III- Diabetic + STD (Glipizide 10mg/kg)	167.83 ± 4.30 ns	182.50 ± 2.26 b***	8.03
IV- Diabetic + GP(400mg/kg)	165.66 ± 3.69 ns	175.66 ± 2.29 a**	5.69
V- Diabetic + GP(800mg/kg)	162.83 ± 2.31 b*	180.50 ± 2.39 b***	9.78

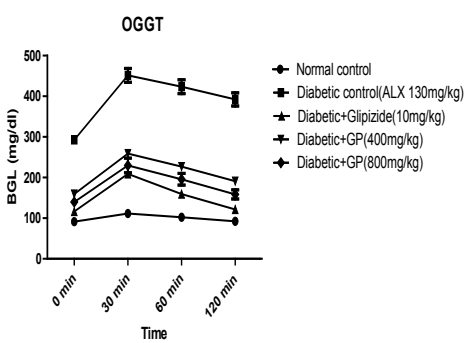
All values given in average body weight (g) shown are mean ± SEM and n=6 Data was analyzed using one way ANOVA and Dunnett's t-test. a=P<0.001,b=P<0.01,ns=non significance

Fig:1Showing blood glucose levels (mg/dl) (Mean ± SEM),



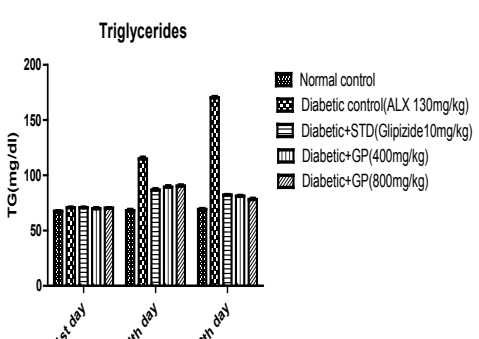
(n=6) in different groups of rats compared to normal control.

Fig:2 Showing blood glucose levels (mg/dl) (Mean ± SEM),



(n=6) in different groups of rats compared to normal control.

Fig:3 Showing triglyceride levels (mg/dl) (Mean ± SEM), (n=6) in



different groups of rats compared to normal control.

Fig:4 Showing total cholesterol levels (mg/dl) (Mean ± SEM), (n=6) in different groups of rats compared to normal

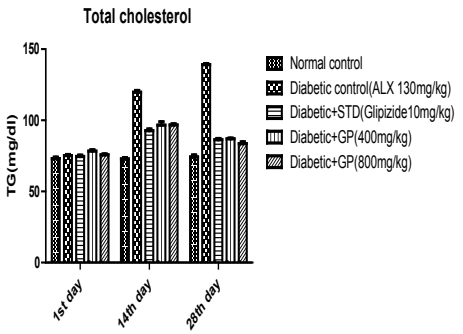


Fig:5 Showing HDL-c levels (mg/dl) (Mean ± SEM), (n=6) in different groups of rats compared to normal control.

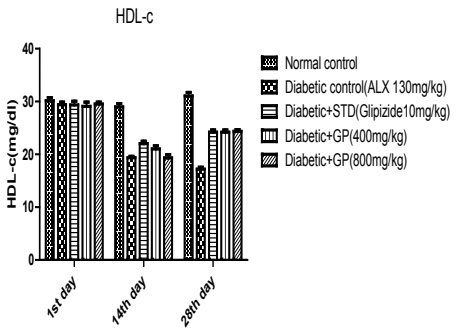


Fig:6 Showing LDL-c levels (mg/dl) (Mean ± SEM), (n=6) in different groups of rats compared to normal control

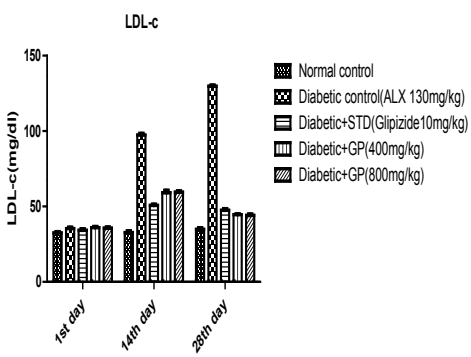


Fig:7 Showing SGOT levels (U/L) (Mean ± SEM), (n=6) in different groups of rats compared to normal control.

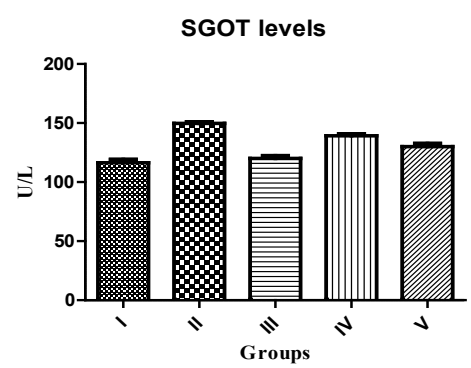


Fig:8 Showing SGPT levels (U/L) (Mean ± SEM), (n=6) in different groups of rats compared to normal control.

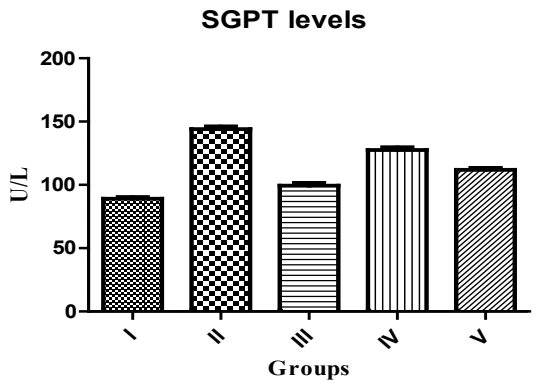
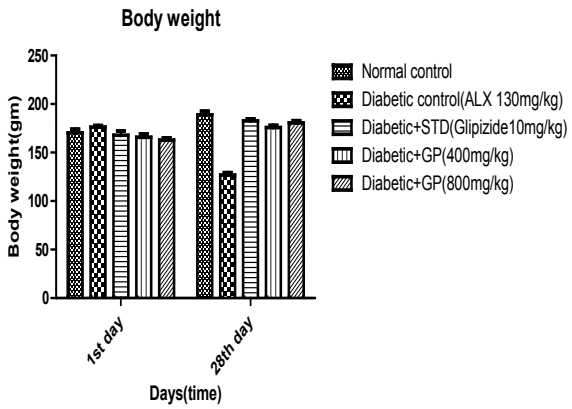


Fig:9 Showing body weights (gm) (Mean ± SEM) (n=6) in different groups of rats



destruction by alloxan may be the primary cause of the recovery. As the ALX (130 mg/kg, i.p.) treated diabetic rats exhibited clear cut abnormalities in lipid metabolism as evidenced from the significant elevation of serum TG, TC, LDL-c, and reduction of HDL-C levels. Treatment with EEGP for 28 days was sufficient to produce a significant reduction in the TG, TC, LDL-c, and significant increase in HDL-c levels in diabetic rats. These results indicate that EEGP has a lipid lowering effect on the diabetic rats.

Loss in body weight was observed in alloxan-induced diabetic rats and was controlled by treatment with Glycosmis pentaphylla fraction. Administration of GP to diabetic (Group IV and V) rats resulted in an increase in body weight compared to diabetic rats (Group II). Results suggested that ethanolic extract of the Glycosmis pentaphylla treatment has positive effect on maintaining body weights in diabetic rats. The protective effect of plant fraction on body weight of diabetic rats may be due to its ability to reduce hyperglycemia. A gradual increase in body weights of Glipizide treated groups (Group III) was similar to that of normal control rats. Alloxan induced diabetes was characterized by severe loss of body weight due to increased muscle wasting in diabetes.

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