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HYPOGLYCEMIC AND ANTIDIABETIC ACTIVITY OF TUBEROUS ROOTS OF *CYANOTIS CRISTATA* IN NORMAL AND ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The present study was aimed to verify the claims and for exploring the hypoglycemic and antidiabetic activity of tuberous roots of *Cyanotis cristata* in normal and Alloxan induced diabetic rats. Alcoholic and aqueous extracts of tuberous roots of *Cyanotis cristata* were prepared and given individually, orally at different doses to different groups of rats fasted for18 h. The serum glucose levels were estimated initially at 0 h (before treatment) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h after the treatment. The alcoholic extract of tuberous roots of *Cyanotis cristata* (ALETRCC) at higher dose (320mg/kg) produced maximal serum glucose lowering effect in both normal and alloxan induced diabetic rats. The aqueous extract of tuberous roots of *Cyanotis cristata* (AQETRCC) produced maximal percent reduction in serum glucose levels with higher dose (400mg/kg). ALETRCC and AQETRCC produced hypoglycemic and antidiabetic activities at 4 h, in a dose dependent manner. The effect produced by ALETRCC was found better than that of standard metformin (45mg/kg) an oral hypoglycemic agent. The ALETRCC has exhibited higher and better hypoglycemic and anti diabetic activity for a prolonged period than that of the AQETRCC. The study results suggested that tuberous roots of *Cyanotis Cristata* possess potential anti-diabetic activity.

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia associated with impairment in the metabolism of carbohydrates, lipids and proteins to a greater or lesser extent. DM is one of the oldest diseases known to man, reported in Egyptian manuscript about 3000 years ago [(1)]. In recent years, there is reportedly a sharp increase in the number of individual's sufferings with diabetes. The International Diabetes Federation reported that, the number was increased from 19 million in 1995 to 66.8 million in 2015. These figures are predicted to increase to 123.5 million by 2040. DM is a major cause of blindness, kidney failure, heart attacks, Stroke and lower limb amputation. Management of diabetes without any side effect is still a challenge to the medical community. There is continuous search for alternative drugs; hence it is prudent to look for options in herbal medicines for diabetes as well. The WHO expert committee on diabetes has listed as one of its recommendations that traditional methods of treatment for diabetes should be further investigated [(2)]. *Cyanotis cristata* (*C. cristata*, *Commelinaceae*) [(3 & 4)]. A prostrate herb with slender, fibrous roots. Traditionally the root is used to reduce the symptoms or cure the Diabetes and for the

relief of swelling and snakebite. The traditional practitioners, folklore herbalists and local tribes of Tirumala Hills region (EasternGhats of Andhra Pradesh state, AP, India) claims that tuberous herbs are of great use in controlling the DM. There are about 248 species, listed in the Flowering Plants of Chittoor District for treating diabetes. Though some of the plants are reputed in the indigenous systems of medicine for their activities, it requires scientific evaluation. The survey of literature revealed that, no systematic and scientific studies have been carried out on *Cyanotis cristata* (*C. cristata, Commelinaceae*). Hence in the present study attempts are made to investigate the hypoglycemic and antidiabetic effects of different doses of alcoholic and aqueous extracts of roots of *C. cristata* in normal and Alloxan induced diabetic rats.

MATERIALS AND METHODS

Collection of plant material

The roots of *Cyanotis cristata* were collected from their natural habitats i.e. Jagamarla reserve forest near Palamaner, in Tirupati, Chittoor District, AP, India, and was authenticated by Dr. K. Madhava chetty (Assistant Professor, Sri Venkateswara University, Chittoor district, A.P).

The Voucher specimens for *Cyanotis cristata* (BCP/2018/1) have been deposited at Bapatla College of Pharmacy, Bapatla, Guntur Dist., Andhra Pradesh. The roots were washed thoroughly to remove adhering soil and earthy matter, sliced to thin chips, dried under shade at room temperature and ground to optimal coarse powder.

Preparation of extracts

The above powdered material was subjected to a successive solvent extraction with petroleum ether (40-60^{0}c), chloroform, alcohol (95%) and chloroform water as solvents. Extraction was carried out for 16 h with each solvent by soxhlet extractor. The yield of extracts was as follows, petroleum ether (0.48%w/w), chloroform (0.98 %w/w) alcoholic (13.10 %w/w) and aqueous (28.18 %w/w). As the bitter principles are reported to have antidiabetic activity and in most of the cases bitter principles are soluble in polar solvents, hence the alcohol and aqueous extracts of roots of *Cyanotis cristata* were screened pharmacologically for hypoglycemic and antidiabetic activity.

Animals

Albino rats (Wistar strain) of either sex weighing between 150 – 200 g were procured from Animal house of Bapatla College Of Pharmacy, Bapatla. These animals were housed in standard environmental conditions in polyethylene cages (20x25x35cm) maintained at controlled room temperature (27±2^{0}c), relative humidity (45-55%) and light/dark cycle (12 : 12 h) and fed with standard commercial rat pellet diet (Amrut Laboratories, Pranav Agro industries Ltd. Sangli, India) and water *ad libitum*. The animals were acclimatized to the laboratory conditions. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals (Reg. number: IAEC/XII/12/BCOP/2018) and approved by the Institutional Animal Ethical Committee (IAEC), of Bapatla College Of Pharmacy, Bapatla, AP, India.

Toxicity studies

The oral acute toxicity (LD_{50}) of the extract was performed by using Albino mice of either sex weighing between 16 - 25 g as per the OECD guidelines No 425 [(5)] by fixed dose method. The acute toxicity (LD_{50}) of alcoholic extract of roots of *Cyanotis cristata* was found to be 1600 mg/kg. Therefore 1/20th (80 mg/kg), 1/10th (160 mg/kg) and 1/5th (320 mg/kg) doses were selected for further study. Whereas the LD_{50} of aqueous extract of *Cyanotis cristata* roots was found to be 2000 mg/kg. Therefore 1/20th (100 mg/kg), 1/10th (200 mg/kg) and 1/5th (400 mg/kg) were selected as lower, medium and higher doses respectively for evaluation of hypoglycemic and antidiabetic activity.

Induction of diabetes [(6-9)]: Alloxan (Otto chemi Ltd., Mumbai, Maharashtra, India)

was freshly prepared by dissolving in distilled water just before use. Diabetes was induced in 18 h fasted Albino rats (Wistar strain) of either sex (150 – 200 g) by intraperitoneal injection of Alloxan (120 mg/kg). The rats were then given 5 % w/v glucose solution in feeding bottles for the next 24 h to prevent hypoglycemia. After 72 h, rats with marked hyperglycemic fasting blood glucose > 250 mg/dl were selected and used for the study. All the animals were allowed free access to water and pellet diet and maintained at standard husbandry conditions.

EXPERIMENTAL DESIGN: Different groups of rats were used for studying the hypoglycemic and antidiabetic effects of alcoholic extract of roots of *Cyanotis cristata* (ALETRCC) and aqueous extract of roots of *Cyanotis cristata* (AQETRCC). The rats were divided into 16 groups consisting of six rats in each group.

Group1: Normal rats treated with vehicle control (0.5 ml), p.o.

Group2: Normal rats treated with Metformin (45 mg/kg), p.o..

Group3: Normal rats treated with 80 mg/kg of ALETRCC, p.o.

Group4: Normal rats treated with 160 mg/kg of ALETRCC, p.o.

Group5: Normal rats treated with 320 mg/kg of ALETRCC, p.o.

Group6: Normal rats treated with 100 mg/kg of AQETRCC, p.o.

Group7: Normal rats treated with 200 mg/kg of AQETRCC, p.o.

Group8: Normal rats treated with 400 mg/kg of AQETRCC, p.o.

Group9: Diabetic rats treated with vehicle control (0.5 ml), p.o.

Group10: Diabetic rats treated with Metformin (45 mg/kg), p.o.

Group11: Diabetic rats treated with 80 mg/kg of ALETRCC, p.o.

Group12: Diabetic rats treated with 160 mg/kg of ALETRCC, p.o.

Group13: Diabetic rats treated with 320 mg/kg of ALETRCC, p.o.

Group14: Diabetic rats treated with 100 mg/kg of AQETRCC, p.o.

Group15: Diabetic rats treated with 200 mg/kg of AQETRCC, p.o.

Group16: Diabetic rats treated with 400 mg/kg of AQETRCC, p.o.

All the animals were subjected to fasting for 18 h prior to experimentation and during the course of time the animals had free access to water. Different doses of both the extracts were dissolved in distilled water and administered orally by gastric intubation, using a force feeding needle to the above mentioned groups of rats respectively. Groups 1 and 9 were served as normal and diabetic controls and received 0.5 ml vehicle (distilled water). Groups 2 and 10 received Metformin (45 mg/kg) orally. Blood samples were withdrawn from the tail vein initially at 0 h (before the treatment) and once again at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h time intervals after the treatment. The collected blood samples were centrifuged (3000 rpm for 20 min) to get clear serum. The separated serum was used for estimation of glucose levels by GOD/POD method [(10)] using commercial glucose kit (M/S Excel diagnostics Pvt. Ltd., Pragathi nagar, Hyderabad, Telangana state, India).

Statistical analysis: Results were expressed as mean±SD (n=6) and data were analyzed by one way ANOVA followed by Dunnett's test. The level of significance was setup at $p < 0.05$*, 0.01** and 0.001*** respectively.

RESULTS: The ALETRCC and AQETRCC at different dose levels produced maximum percentage reduction in serum glucose levels at 4 h in normal and alloxan induced diabetic Albino rats. Whereas the standard drug Metformin (45 mg/kg) produced response at 6 h.

Table: 1 % Reductions in serum glucose levels of normal albino rats treated with ALETRCC and AQETRCC

Groups →	Control (distilled water)	Metformin	Alcoholic extract of roots of *C.cristata*			Aqueous extract of roots of *C.cristata*		
Dose(mg/kg) →	0.5 (ml)	45	80	160	320	100	200	400
Time (h)↓	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
0	-	-	-	-	-	-	-	-
0.5	-0.14±1.58	11.02 ± 0.15**	1.67±1.89	3.68±3.35	4.51±4.21	0.14±1.57	2.13±2.37	3.39±0.86
1	0.11±0.87	19.86 ± 0.39**	9.44±3.07	15.25±2.12	16.61±2.67	5.57±1.19	9.29±3.42	11.43±1.68
2	0±1.23	23.24 ± 0.19**	20.83±2.19	26.61±2.95	30.44±4.02	14.35±2.33	21.33±2.27	20.80±2.03
3	1.11±1.19	26.04 ± 0.27**	30.16±1.62	36.23±3.29	42.25±2.72	18.89±1.31	25.46±2.14	26.10±2.20
4	0.07±1.50	35.57 ± 0.23**	31.32±1.19	38.89±2.07	43.49±2.59	23.21±2.26	31.68±1.13	38.84±1.41
6	-0.11±1.11	37.51± 7.97**	27.49±3.61	36.30±3.33	39.55±2.48	13.22±1.47	21.18±2.86	17.82±3.39
8	1.12±2.43	19.86 ± 0.31**	22.56±2.28	34.43±2.50	36.12±3.61	8.27±1.89	16.27±2.39	10.91±2.25
12	0.18±0.85	10.45 ± 0.28**	20.63±1.91	29.23±3.34	32.99±3.83	2.92±1.55	8.74±2.95	7.32±3.19
16	-0.09±1.60	7.52 ± 0.16**	9.41±2.57	22.33±3.36	24.89±2.93	1.94±1.50	4.01±2.78	4.92±2.62
20	2.53±0.94	4.39 ± 0.25	7.37±2.47	11.79±3.09	20.21±2.59	0.81±1.77	0.86±3.01	1.87±3.55
24	0.63±1.37	1.90 ± 0.19**	4.54±2.98	7.84±3.76	14.75±2.53	0.09±0.95	0.86±3.30	1.08±2.14

n=6 significant at p<0.05*, 0.01** and 0.001***.

Table: 2 % Reductions in serum glucose levels of Alloxan induced diabetic albino rats treated with ALETRCC and AQETRCC

Groups →	Control (distilled water)	Metformin	Alcoholic extract of tuberous roots of *C. Cristata*			Aqueous extract of tuberous roots of *C. cristata*		
Dose (mg/kg) →	0.5 (ml)	45	80	100	320	100	200	400
Time (h)↓	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
0	-	-	-	-	-	-	-	-
0.5	0.63±0.33	15.81 ± 0.15**	3.15±2.72	6.76±2.98	7.11±1.76	2.62±1.95	2.94±2.50	6.62±3.85
1	0.19±0.52	16.78 ± 0.49**	13.00±3.11	20.71±2.75	21.85±3.06	11.03±2.75	12.46±2.67	19.82±2.30
2	0.47±0.73	22.85 ± 0.03**	23.73±2.78	32.69±2.59	32.91±1.65	21.99±2.98	20.41±1.61	29.51±4.49
3	1.08±1.02	30.25 ± 0.91**	30.98±0.52	39.52±2.86	44.81±1.40	25.79±1.65	24.73±3.03	35.48±1.84
4	0.48±0.65	38.91 ± 0.20**	33.23±2.08	44.28±2.87	47.37±1.74	28.83±0.80	34.16±0.74	41.13±0.65
6	0.58±0.57	41.39± 1.10**	28.93±0.43	38.53±2.79	42.25±2.06	21.82±2.06	20.77±2.24	29.01±2.77
8	-0.20±1.02	32.34 ± 0.53**	26.79±2.95	35.21±0.94	39.82±2.29	16.72±1.75	16.80±1.64	25.58±1.49
12	0.22±0.59	23.82 ± 0.34**	21.13±2.86	31.92±1.97	38.97±0.98	10.10±2.47	11.77±1.65	17.86±2.88
16	0.58±0.70	16.08 ± 0.50**	16.42±0.69	26.57±2.95	29.91±2.70	6.17±1.85	7.32±2.16	12.44±2.60
20	0.14±0.90	7.30 ± 0.12**	14.54±1.75	19.93±1.93	24.29±2.03	3.88±2.89	3.10±1.64	6.86±1.95
24	0.58±0.61	0.96 ± 0.06**	10.30±1.27	15.45±1.82	15.10±2.62	1.21±2.66	1.48±2.06	2.85±2.7

n=6 significant at p<0.05*, 0.01** and 0.001***.

Figures: Hypoglycemic and antidiabetic activity of tuberous roots of *cyanotis cristata* in normal and alloxan induced diabetic rats

% Reductions in serum glucose levels of normal albino rats treated with ALETRCC

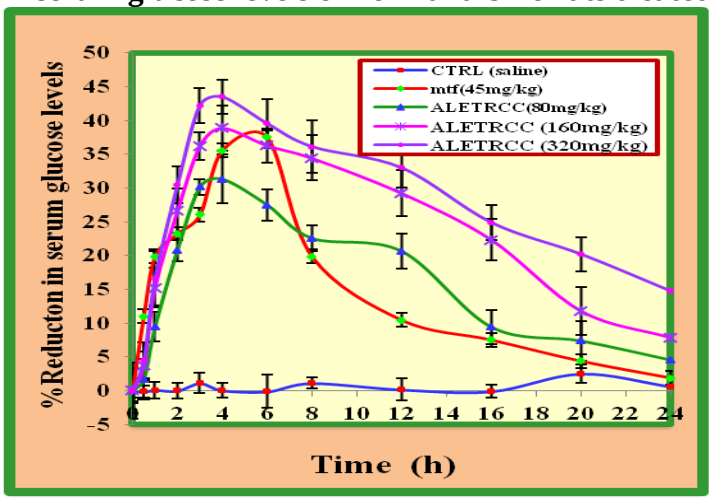

Fig. 1: % Reduction in blood glucose levels of normal albino rats treated with ALETRCC

% Reductions in serum glucose levels of normal albino rats treated with AQETRCC

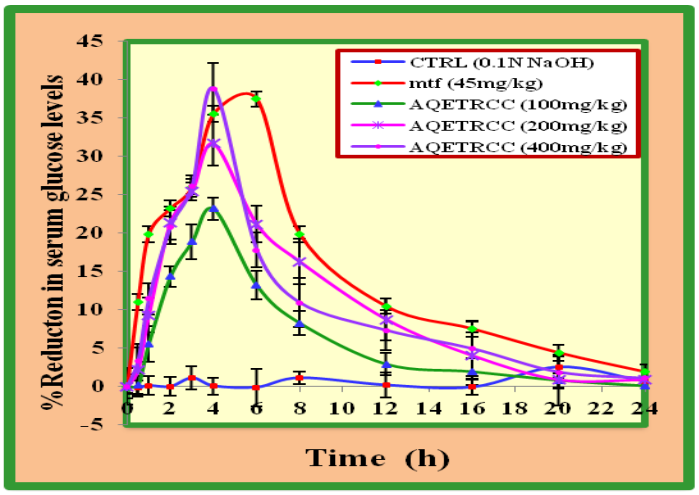

Fig. 2: % Reduction in blood glucose levels of normal rats treated with AQETRCC

% Reductions in serum glucose levels of Alloxan induced diabetic albino rats treated With ALETRCC

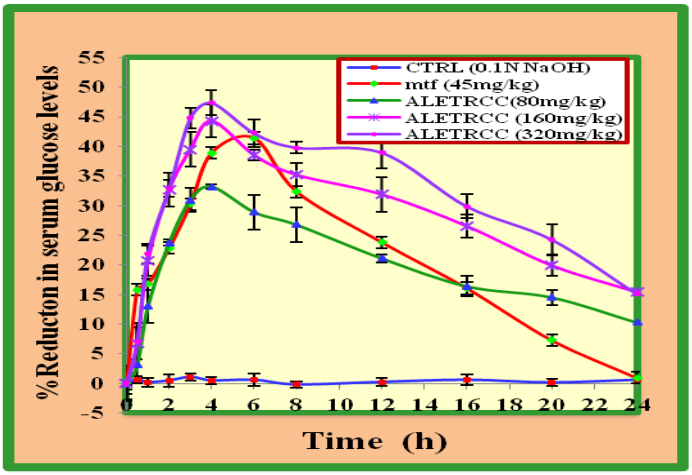

Fig. 3: % Reduction in blood glucose levels of Alloxan induced diabetic albino rats treated with ALETRCC

% Reductions in serum glucose levels of Alloxan induced diabetic albino rats treated with AQETRCC

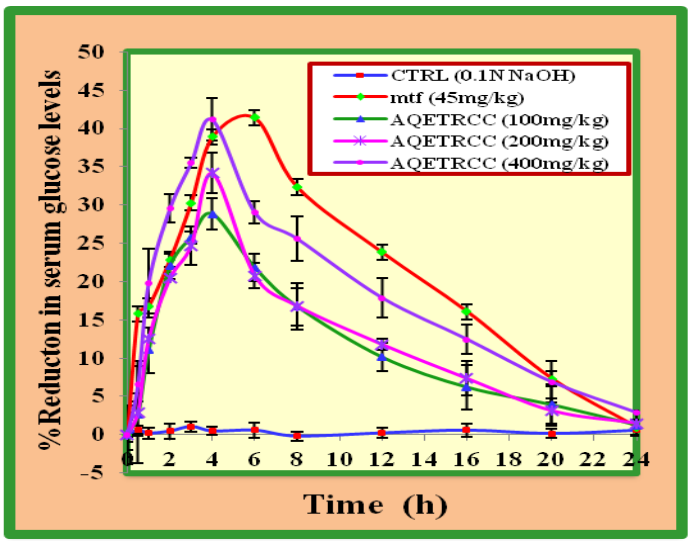

Fig. 4: % Reduction in blood glucose levels of Alloxan induced diabetic albino rats treated with AQETRCC

In normal albino rats, the ALETRCC with different dose levels i.e, (80 mg/kg, 160 mg/kg, 320 mg/kg) has reduced the serum glucose levels by (31.32±1.19%), (38.89±2.07%) and (43.49±2.59%) at 4 h respectively. Similarly AQETRCC with 100, 200 and 400 mg/kg doses has reduced the serum glucose levels by (23.21±2.26%), (31.68±1.13%) and (38.84±1.41%) at 4h respectively. Metformin (45 mg/kg) has produced percentage reduction in serum glucose levels by 37.51 ± 7. 97% at 6 h in normal healthy Albino rats. The detailed results of the percentage reduction in serum glucose levels of ALETRCC and AQETRCC in normal rats are compiled in table 1 and figures 1 and 2 respectively. In Alloxan induced diabetic rats, the ALETRCC with different dose levels i.e, (80mg / kg, 160 mg / kg, 320 mg / kg) has reduced the serum glucose levels by (33.23±2.08%) , (44.28±2.87%) and (47.37±1.74%) at 4 h respectively. Similarly, AQETRCC at 100, 200 and 400 mg / kg doses have reduced the elevated serum glucose levels by (28.83±0.80%), (34.16±0.74%), (41.13±0.65%) at 4 h respectively. Metformin (45 mg / kg) has produced percentage reduction in serum glucose levels by 41.39 ±1.10 % at 6 h. The

detailed results of the percentage reduction in serum glucose levels of ALETRCC and AQETRCC in alloxan induced diabetic rats are compiled in table 2 and figures 3 and 4 respectively. The hypoglycemic and antidiabetic activity produced by ALETRCC at different doses 160 and 320 mg/kg are comparable and even more than that of the standard drug Metformin (45 mg/Kg), whereas the hypoglycemic and antidiabetic activity at higher dose (400 mg/kg) of AQETRCC has produced similar effect to that of standard drug Metformin (45 mg/Kg).

DISCUSSION

In the present study, the hypoglycemic and antidiabetic activity of vehicle, Metformin (45 mg/kg), different doses of ALETRCC (80, 160 and 320 mg) and AQETRCC (100, 200, and 400 mg/kg) were evaluated in normal and Alloxan induced diabetic rats. In normal and Alloxan induced diabetic Albino rats, single dose treatment of Metformin produced reduction in serum glucose levels at and 6 h. The hypoglycemic and antidiabetic activity produced by different doses of ALETRCC at 160 and 320 mg/kg are comparable and even more than that of the standard Metformin (45 mg/Kg). Whereas the ALETRCC at 80 mg/kg produced mild reduction in serum glucose levels than that of metformin (45 mg/kg). The AQETRCC at 400 mg/kg produced a good reduction in serum glucose levels at 4 h. whereas the hypoglycemic and antidiabetic activity of AQETRCC at 100 and 200 mg/kg was found to be lower and non significant than that of metformin (45 mg/kg). The ALETRCC has higher and better hypoglycemic activity than that of AQETRCC in normal and Alloxan induced diabetic albino rats.

CONCLUSION

From this study it can be concluded that ALETRCC possess beneficial effects on serum glucose levels in normal and Alloxan induced diabetic Albino rats. Further pharmacological and biochemical investigations are under progress to elucidate the mechanism of the hypoglycemic and antidiabetic effect of extract.

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