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RESEARCH ARTICLE

ANTIDIABETIC ACTIVITY IN AQUEOUS LEAF EXTRACT OF CICERACIDA LINN IN ALLOXAN INDUCED DIABETES RATS MODEL

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ABSTRACT

Diabetes mellitus is the most common endocrine disorder that impairs glucose homeostatis resulting in severe diabetic complication including retinopathy nephropathy, angiopathy, neuropathy due to perturbation in utilization of glucose. The present study was focused on antidiabetic effect of aqueous leaf extract of *ciceracida* (ALEC) in alloxan induced diabetic rats using Glibenclimide as a standard. The aqueous leaves extract of *ciceracida* administered at a dose of 100 mg/kg, 250 mg/kg and 500 mg/kg orally to the diabetic rats for 10 days in acute treatment group, whereas sub acute treatment for 30 days administration was done. In acute treatment group blood glucose level investigation was carried out on 4th, 7th and 10th day similarly in sub acute study animals were tested for blood glucose level on 14th, 21th and 30th day. Both acute and sub acute treatment groups shown reduction in elevated blood glucose level but sub acute treatment groups shown noticeable significant reduction in blood glucose level as compared with the control group. The study concluded that ALEC can be a new clinical significant choice in the treatment of diabetes.

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INTRODUCTION

Diabetes mellitus (DM), often simply referred to as diabetes, it is a group of metabolic disorder in which a patient has high blood sugar, either because the body does not produce enough insulin, or because beta cells do not respond to the insulin secretion. This high blood sugar produces the classical symptoms of polyuria, polydipsia and polyphagia. It is characterized by hyperglycaemia due to defective insulin action, insulin secretion or both[1]. Globally rates of type 2 diabetes were 15.1 million in 2000[2]. The number of people with diabetes worldwide is projected to increase to 36.6 million by 2030[3]. In 2007, 23.6 million people, or 7.8% of the United States population had type 2 diabetes According to the World Health Organization (WHO), there are approximately 160,000 diabetics worldwide, the number of diabetics has double in the last few years and is expected to double once again in the year 2025[4,5]. Due to its high prevalence and potential deleterious effect on a patient physical and psychological state, diabetes is a major medical concern[6].

World Health Organization (WHO) has suggested the evaluation of the potential of plants as effective therapeutic agents, especially in areas in which we lack safe modern drugs. Therefore, the search for the more effective and safer anti-hyperglycaemia agent becomes an area of active research.

Herbal drugs are mostly out of toxic or of less toxic with fewer side effects compared to the synthetic drugs. Hence, there is persistent interest all over the world to explore other alternative therapies which are believed to be effective, safer and economical[7].

Cicericida belogs to family euphorbiaceace is an Indian medicinal plant which is extensively used in Ayurveda and other alternative system of medicine. it is widely available in the northeast region of India specially in assam[9] Keeping these facts in view, the present study was undertaken to prove its antidiabetic activity in alloxon induced diabetic rat model.

MATERIALS AND METHODS

Collection of Plant materials

Leaves of *ciceracida* linn were collected from Bongaigaon distict of Assam and taxonomi were authenticated by Dr. B Doley Department of botany Goalpara College. Goalpara Assam. Leaves was washed under tap water and were efficient dried in sun for 7 days, and were stored in polythene bag in cool place.

Preparation of Aqueous Extraction

Leaves of *ciceracida* were sun dried, coarsely powdered and extracted by soxhelet hot extraction with water for 48 hours.

Preliminary Phytochemical screening[9]

About 50 mg of the solvent-free extract was stirred with little quantity of dilute HCl and then filtered. The filtrate was tested for presence of various phytochemical constituents such as alkaloids, Carbohydrates, Steroids, Phenols, Tannins, Flavonoids, Glycosides and Saponins. Samples was analysed with HPLTC for chemical identification.

Drugs and chemicals

Alloxan monohydrades (Intas pharmaceutical) standard drugs Glibenclimide (Intas pharmaceutical pvt) were of analytical grade,

Preparation of alloxan solution

Alloxan was prepared freshly by dissolving 120 mg/kg of alloxan in Na- citrate buffer (0.01 M, Ph 4.5) and maintaned on ice prior to use the injection volume was 0.2 ml.

Experimental Animals

Experiments were performed with wistar rats, weighing about 180-200 gm. The animals were housed in individual polypropy-lene cages under standard laboratory conditions of light, temperature (22 ± 1°C) and relative humidity for at least one week before the beginning of experiment, to adjust to the new environment and to overcome stress possibly incurred during transit. Animals were given standard rat pellets and drinking water add libitum. The animals were fasted 12 hours before the conduct of experiment and during the experiment they were withdrawn from food and water.

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling techniques were employed in this study.

The animal were kept fasting for overnight providing only water. Then the extract (ALEC) was administered orally at the dose of 2000 mg/kg. The animals were observed for toxic symptoms and behavioral changes continuously for the first 4 hrs after dosing. Finally, the number of survivors was noted after 24 hrs. From the next day onwards, each day 1 hour the behavioral change, clinical symptoms or mortality was observed in the same animals for the next 14 days. Aqueous leaves extract of *ciceracida* were shows no mortality in rats. No toxic symptoms were observed even at the dose of 2000 mg/kg during the period of 14 days. The LD 50 value of ALEC was found to be more than 2000 mg/kg, so dose were selected randomly as 100 mg 250 mg and 500 mg.

Experimental Design[10,11,12]

Diabetes was induced by using Alloxan monohydrades 120 mg/kg is dissolved in chilled normal saline and given intraperitoneal to overnight fasted animals. The rats were kept for the next 24 hours on 10% of glucose solution to prevent hypoglycemia and death. After 48 hours of inducing fasting blood glucose levels were measured. The animal which did not shown blood glucose level of more than 250 mg/dl were rejected.

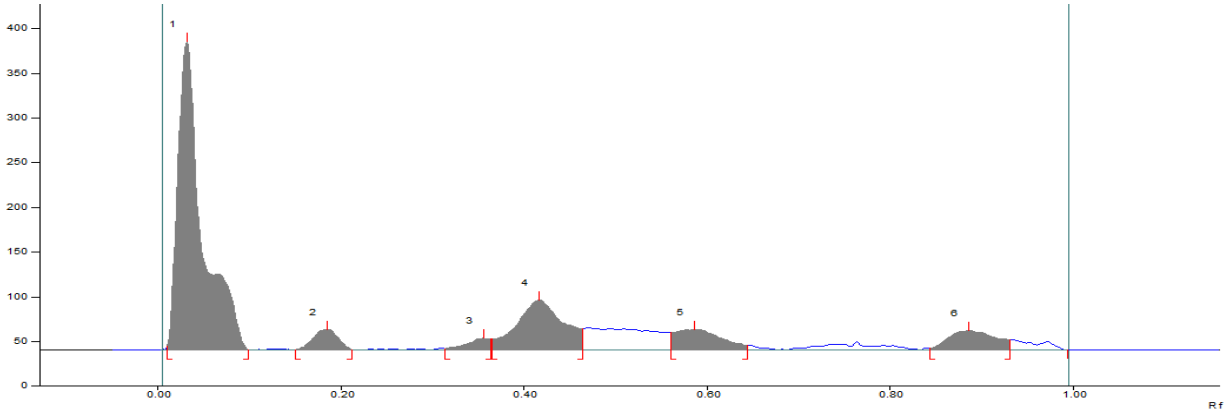
In acute study 36 animals were selected for the experiment was divided into six animal in six groups. Group A are kept separately as a normal control. Remaining 30 animals are made diabetic by injecting alloxon monohydrates at a dose of 120 mg/kg i.p. the rats were provided 10% glucose solution bottles in their case for next 24 hours to reduced hypoglycemia. The blood glucose were measured after and before 72 hours of alloxan injection to confirmed the diabetes.

Group- A: Animals are served as control and received 1% w/v sodium CMC.

Table A R_f values

Track 6, ID: Cicaraeida

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	4.2 AU	0.03 Rf	344.6 AU	71.59 %	0.10 Rf	0.2 AU	6603.1 AU	60.05 %
2	0.15 Rf	0.2 AU	0.19 Rf	23.1 AU	4.80 %	0.21 Rf	0.2 AU	452.8 AU	4.12 %
3	0.31 Rf	1.5 AU	0.36 Rf	13.0 AU	2.69 %	0.36 Rf	12.3 AU	242.7 AU	2.21 %
4	0.37 Rf	12.4 AU	0.42 Rf	56.0 AU	11.63 %	0.46 Rf	23.6 AU	2037.8 AU	18.53 %
5	0.56 Rf	19.5 AU	0.59 Rf	23.0 AU	4.78 %	0.65 Rf	4.9 AU	855.5 AU	7.78 %
6	0.85 Rf	2.3 AU	0.89 Rf	21.7 AU	4.51 %	0.93 Rf	10.9 AU	804.9 AU	7.32 %



Graph A- R_f values

Group- B: Diabetic control treated animal receiving only 1% sodium CMC
Group- C: Standard, anim als are treated with Glibenclimide at a dose of 10 mg/kg.
Group- D: Diabetes induced animal were treated with ALEC 100 mg/kg.
Group- E: Diabetes induced animal were treated with ALEC 250 mg/kg.
Group- F: Diabetes induced animal were treated with ALEC 500 mg/kg.

ALEC administration was carried for 10 days blood glucose level estimation was investigated on 4th 7th and 10th day. Similarly as above sub acute grouping was made and the study duration was for 30 days, and blood glucose level was investigated on 14th 21th and 30th day.

Biochemical analysis

Blood Samples were collected from the animal prior to the treatment with above schedule and after 30 min of Glibenclimide administration 10th and 30th day. Blood samples obtained from the retro orbital venus plexes of rats under the ether anesthesia using a glass capillary tube and was centrifuged 2500 rpm for 10 mints. To separates serum. The serum was used for biochemical analysis of blood glucose levels.

Statistical analysis

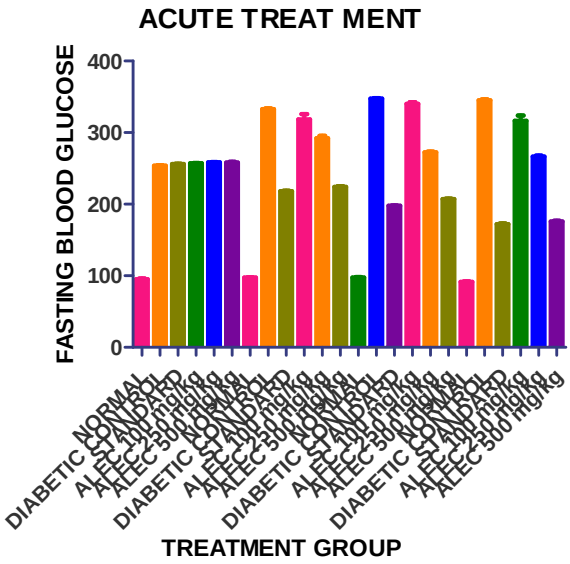
Data was expressed as mean ± S.D (n=6). Statistical analysis was done using ANOVA, followed by Dunnet's multiple comparisons. Values are considered statistically significance when at p<0.001.

RESULTS

HPTLC was performed in S.D.M. centre for research in ayurveda and allied sciences (ayush centre for excellence and recognized SIROS by DSIR) laxminarayana nagar, p.o. kuthpady –udupi [karnataka]. R_f values were identified and matched with library reference values. R_f library values suggest that presence of flavonoids (querecin -3-o rutinosides, isoquerecin)

In the acute study group the effect of ALEC was studied to its ant diabetic activity, alloxan was used an diabetic inducing agent. A marked rise in fasting blood glucose level observed in diabetic control compare to normal controls rat. Aqueous leaf extract of *ciceracida* at a dose 100, 250 and 500 mg/kg exhibited a dose dependent significant anti-hyperglycemic activity on 4th 7th and 10th day post treatment. But anti-hyperglycemic effect of aqueous extract of *ciceracida* at was found less effective than the reference standard drugs is Glibenclamide. In the sub acute study group the effect of ALEC was studied for its anti diabetic activity using alloxan as an diabetic inducing agent.

A marked rise in fasting blood glucose level observed in diabetic control compare to normal controls rat. Aqueous leaf extract of *ciceracida* at a dose 100, 250 and 500 mg/kg exhibited a dose dependent significant anti-hyperglycemic activity on 14th 21th and 30th day post treatment. The extract dose of 500 mg/kg caused reduction in blood glucose level. But anti-hyperglycemic effect of aqueous extract of *ciceracida* at was found less effective than the reference standard drugs is Glibenclamide.



Graph 1 Acute treatment group

Table 1 Effect of aqueous leaf extract of *ciceracida* on fasting blood glucose level in alloxan induced diabetes rat model Alloxan 120 mg/kg Glibenclamide 10 mg/kg

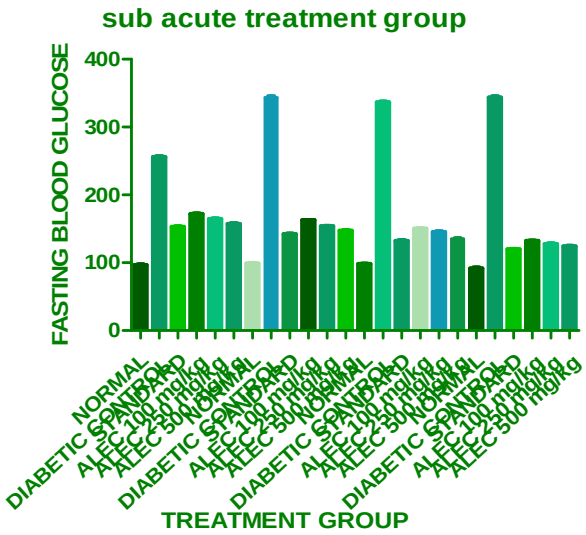
		Fasting blood glucose level in mg/kg			
Group	**Treatment**	**Basal value**	**4TH DAY**	**7TH DAY**	**10TH DAY**
A	Normal control	95.17 ± 2.31	97.50 ± 1.04	97.67 ±1.50	91.33± 1.66
B	Diabetes control	253.8± 1.47	332.8± 2.31	347.5±1.87	345± 3.47
C	Standard	256.0±1.54	218.8±1.72***	197.8±1.16***	172.3±2.06***
D	Alloxan + ALEC 100 mg/kg	256.7±1.21	318.0±11.05*	340.2±6.04	316.7±17.51**
E	Alloxan + ALEC 250 mg/kg	258.3±1.21	292.7±6.91**	272.0±2.28**	266.3±3.50**
F	Alloxan + ALEC 500 mg/kg	258.2±2.40	224.2±2.63***	207±2.28***	175.8±2.13***

Values are mean ± S.D: n= 6, *P< 0.05, **P < 0.01 and ***P < 0.001 vs diabetic scontrol

Table 2 Effect of aqueous leaf extract of *ciceracida* on fasting blood glucose level in alloxan induced diabetes rat model.

		Fasting blood glucose level in mg/kg			
Group	**Treatment**	**Basal value**	**14TH DAY**	**21TH DAY**	**30TH DAY**
A	Normal control	96.5 ± 2.42	98.83 ± 1.72	98.17±0.75	91.33± 3.67
B	Diabetes control	255.8 ± 2.31	343.7 ± 3.61	336.5±2.25	343.7 ± 3.50
C	Standard	152.7± 2.06	142.2 ± 1.94***	132.3±1.63***	119.8 ±1.16***
D	Alloxan + ALEC 100 mg/kg	171.7 ± 1.96	162.7± 2.65*	150 ±1.26*	132 ± 1.41*
E	Alloxan + ALEC 250 mg/kg	164.5 ± 1.87	153.7 ±0.81***	145 ± 3.16***	127.5 ± 2.07***
F	Alloxan + ALEC 500 mg/kg	157.0 ± 2.53	147.3 ± 1.21***	135 ±1.67***	124 ±1.78***

Values are mean ± S.E.M: n= 6, *P< 0.05, **P < 0.01 and ***P < 0.001 vs diabetic contro



Graph 2 Sub acute treatment group

DISSCUSSION

In the modern era of medicine there are different types of oral hypoglycaemic agents are available along with insulin for the treatment of diabetes, but have generated chemophobias in the patients there is an increased demand by patients to use the natural products with antidiabetic activity[13]. The present study was designed to investigate antihyperglycemic potential and to provide scientific validation to prove antihyperglycemic activity of *ciceracida* linn. Alloxan is known to induce free radical production and cause tissue injury, and the pancreas is especially susceptible to the action of alloxan induced free radical damage.

General phytochemical study reveals that Many flavonoids containing plants serve as a hidden wealth of potentially useful natural products for diabetes control[14]. A naturally occurring flavonoids (-)- epicatechin, chard – *Beta vulgaris* L. var cida, salymarin, *Terminalia catappa* leaves[15], walnut leaves – *Julgaris regia* L,[16] and *Gymnema sylvestre*[17]. used as traditional diabetes managements. The extract appears relatively safe judging from the acute toxicity test. The result of this present study therefore justifies the use of *ciceracida* leaves in the treatment of diabetes mellitus. Flavonoids act by regeneration of previously necrosed pancreatic beta cells, hence re-establishing insulin production[18]. From the present study, it can be concluded that aqueous extract of *ciceracida* leaves some of its phytoconstituents possess significant antihyperglycemic property. Further studies are needed to investigate and elucidate the possible mechanism of action of active ingredients, establish complete safety profiles.

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